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CONSULTANTS BUREAU, INC.

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

PUBLIC HEALTH SERVICE

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August 15, 1958

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PHYSIOLOGY

THE ROLE OF ACETYLCHOLINE IN THE FUNCTIONING OF THE AUTONOMIC NERVE TRUNKS

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(Received May 5, 1957. Presented by Active Member Acad. Med. Sci. USSR M. D. Tushinskii)

Calabro [10] was able to establish that during excitation of the nerve trunks, physiologically active mediating substances are released. Certain authors, such as Nachmansohn [13], minimize the role of acetylcholine to mere depolarization of the cell membrane which produces the action current, and enables it to travel along the nerve fiber. The concept that acetylcholine plays the role of a mere depolarizer of the cell membrane has encountered a whole series of objections [6, 9, 11, 12].

As far back as 1935, A. A. Ukhtomskii [8] suggested the hypothesis that mediators of the nerve impulses play the role of humoral "conditioners" preparing the system for a more adequate utilization of the action currents.

In subsequent studies, the physiological role of acetylcholine was investigated by demonstrating the direct effect of acetylcholine or eserine upon the functional state of the nerve trunks. E. B. Babskii and his co-workers [1, 2, 3] showed that large concentrations of acetylcholine caused a prolongation of both the relative and absolute refractory phases and a diminution of the sensitivity of the nerve trunks to stimulation, while small doses exerted just the opposite effects, namely, increased their functional sensitivity. However, experiments in which the concentration of the mediating substance within the nerve tissue has been artificially increased are still not such as to permit a complete visualization of their physiological significance.

In the present study we attempted to clarify the role played by acetylcholine in the functioning of the autonomic nerve trunks, by means of the method which utilized a temporary destruction of the acetylcholine-forming tissue within the organism. Under such conditions we studied the excitability, refractory states and lability of the nerve trunks.

EXPERIMENTAL METHODS

The experiments were conducted on the individual nerve trunks of the sympathetic and parasympathetic systems in the neck region of the cat. For the purpose of investigating absolute and relative refractory states, as well as the phase of reinforcement we used a special generator of paired impulses, so constructed as to make possible independent variation of the amplitude, duration, and time interval between the pulses. For rhythmic stimulation we generated direct impulses with a frequency of from 3 to 800 cps. The action currents were recorded on photofilm with the aid of a two-channel cathode oscillograph type OB-2. The oscillograph had a range of 0.1 to 2000 cps.

The exposed portions of the stimulating and discharging electrodes (made of silver or platinum) were insulated and so prevented from making contact with the surrounding tissues. The action currents were recorded at a distance of 8 to 12 cm from the stimulating electrodes. A grounded silver plate was placed between the stimulating and discharging electrodes, the purpose being to prevent a short circuit. Experiments were carried out on normal, unoperated cats as well as on animals which had been previously subjected to removal of some 60% of their pancreatic gland, in order to temporarily disturb the acetylcholine-forming mechanism of

the organism. Such an operation was not observed to upset the functions of islet cells in the body. As controls we used operated animals who received after the operation, along with the eserine, 1-2 cc of a 1 :10,000 solution of acetylcholine. This solution was given intramuscularly beginning with the fourth postoperative day, while in many experiments the acetylcholine was given intravenously one hour before beginning the study.

EXPERIMENTAL RESULTS

In unoperated animals the absolute refractory phase in the sympathetic as well as the parasympathetic cervical nerve trunks lasted from 1.5 to 3.8 σ. The relative refractory phase lasted 8 to 18 σ. The phase of reinforcement, determined by the second stimulus having a threshold value, would make itself manifest 12 to 40 σ after the first impulse. When thresholds were being investigated for stimuli of very short duration, namely of the order of 0.5 to 1 σ, it was found that they had to be quite powerful. For this reason we chose stimuli lasting 3-4 σ, this duration being quite adequate for our purposes. The use of direct impulses of such duration

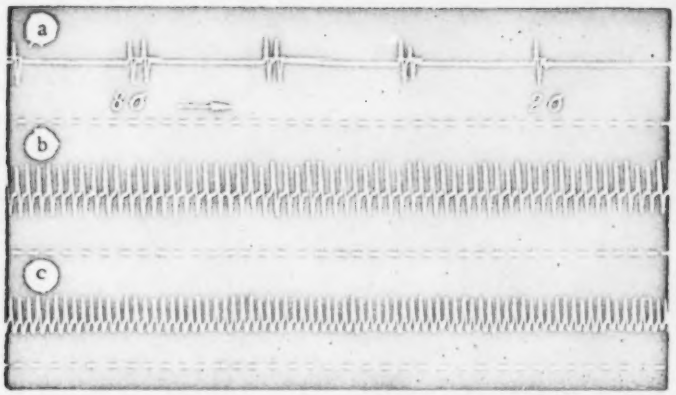

Fig. 1. Action currents in the cervical sympathetic nerve of the cat. Interval between stimuli: a) from 8 to 2 σ. Frequency of stimuli: b) 100 cps; c) 200 cps. Time marker 0.01 second.

produced no alterations in the length of the relative refractory phase. Only when the impulses were lengthened to 5-6 σ could there be noted a change in the time of the relative refractory phase. The threshold of stimulation comprised 4.5-5.5 scale divisions. The maximum stimulatory rhythm which could be carried by the nerve trunks without a labile transformation equaled 200-250 cps. When the frequency of stimulations rose to 300 cps and higher, the rhythm of the action currents became transformed, being accompanied by lower amplitudes of the action currents.

Figure 1,a shows that with intervals between stimuli of 8 σ or less there is observed a lower amplitude from the second stimulus, i.e., the relative refractory phase, which becomes absolute as the interval between the two stimuli is decreased to below 2 σ, there then being no action current produced by the second stimulus. Figure 1,b shows that a frequency of 100 cps gives the highest amplitude of the action currents, while Fig. 1,c demonstrates that increase of the frequency to 200 cps depresses somewhat the amplitude without upsetting the rhythm of stimulation.

The second series of experiments was conducted on 20 cats 6 to 14 days after the major portion of their pancreas had been removed. In previous investigations [4, 5] it had been shown that the greatest disturbances in the acetylcholine balance of the organism occur at this time.

In the operated animals it was found that the absolute refractory phase lengthened to 2.6-6.4 microseconds, compared with the normal 1.5-3.8 microseconds. Also, the relative refractory phase grew from a normal of 8-18 microseconds to 10-25 microseconds.

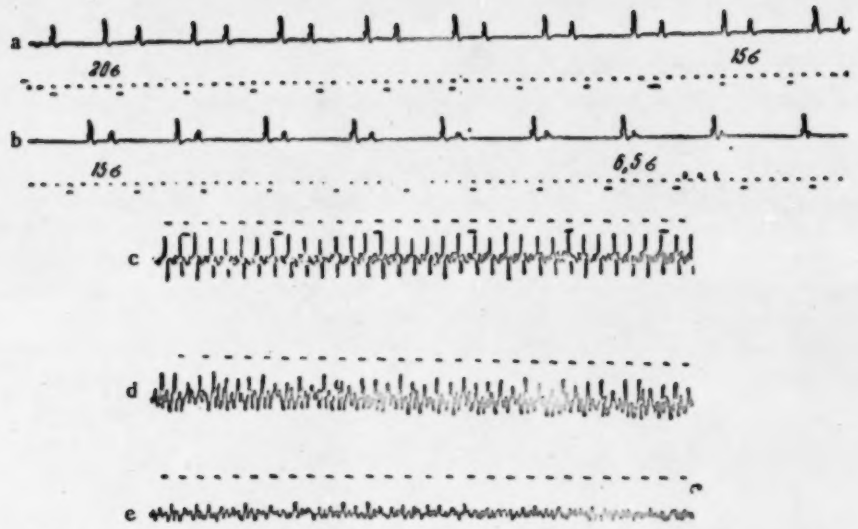

Fig. 2. Action currents in the cervical sympathetic nerve of a cat 8 days following a partial pancreatectomy.
Interval between stimuli: a) from 20 to 15 microseconds; b) from 15 to 6.5 microseconds. Frequency of stimuli: c) 100 cps; d) 125 cps. Time markers (0.01 and 0.05 seconds).

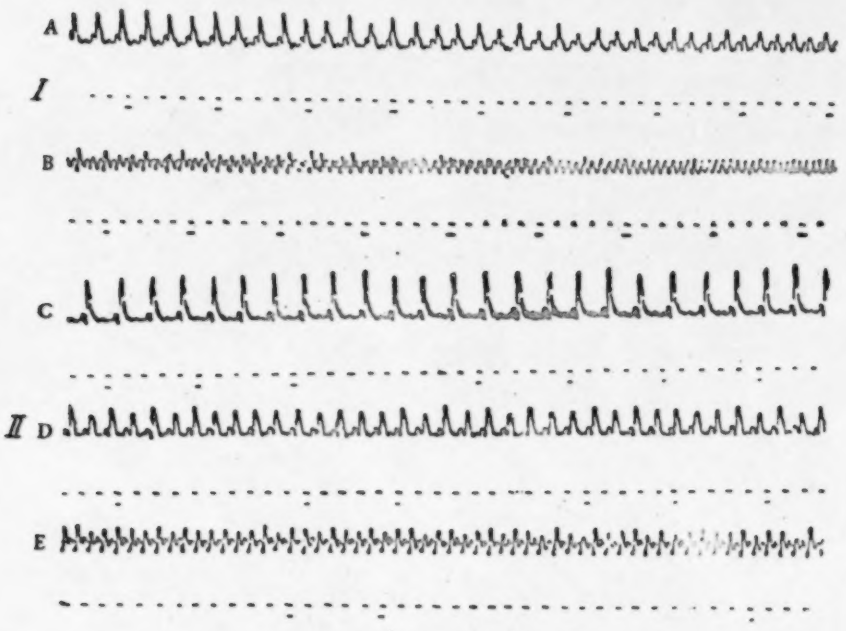

Fig. 3. Action currents in the cervical parasympathetic nerve of the cat 8 days following a partial pancreatectomy.
I) Before introduction of acetylcholine; II) after introduction of acetylcholine.
Frequency of stimulations: B) from 100 to 125 cps;C) 100 cps; D) 125 cps; E) 106 cps.
Time markers 0.01 and 0.05 seconds.

The phase of reinforcement could not be demonstrated in the majority of the experiments. The threshold of stimulation rose by 3-6 scale divisions, while at the same time the stimuli had to be prolonged. The lability of the rhythm fell to 100-110 oscillations, compared to the normal of 200-250 oscillations per second. Stimulation with a frequency of 150 cps would upset the regular rhythm of the action currents and depress the amplitudes up to the point of complete inhibition of the electrical activity of the nerve trunk.

Figure 2 demonstrates the action currents present in the cervical sympathetic nerve of a cat 8 days following the removal of the major portion of its pancreas. The oscillogram in line a shows a diminution of the action current after the second stimulus when the latter follows the first stimulus by 20 microseconds (relative refractory phase), while when the two stimuli are approximate to within 6.5 microseconds of each other, as shown in line b, the action current from the second stimulus disappears altogether (absolute refractory phase). If stimuli have a rhythm of 100 cps, the action currents maintain their regularity as can be seen in line c, but increasing the frequency to 125 cps upsets the rhythm, as can be seen in d, while a further increase to 150 cps leads to infrequent action currents of low amplitude (e).

It can be seen, then, that removal of the major portion of the pancreas affects adversely the functional state of the nerve trunks of the autonomic system. Concretely, these changes consist in a lengthening of the absolute and relative refractory phases, a rise in the threshold of sensitivity, and a depression of the level of lability.

In the succeeding series of experiments the operated animals received daily intramuscular injections of acetylcholine solution with eserine. Under such conditions these experimental animals failed to register any material changes in the state of irritability, length of the refractory phases, or level or lability of the nerve trunks being studied. The absolute refractory phase varied within 2.6 to 5.2 σ. The relative refractory phase was 7.5-14 σ. The reinforcement phase after the first stimulus appeared within 30-40 σ. The threshold of irritability equaled 5-6 divisions of the scale. The maximal rhythm of stimulation, synchronized with the action currents, gave values of 150-200 oscillations per second.

In a series of experiments the operated animals were first tested for the functional state of their nerve trunks and then given a single injection of acetylcholine. The nerve trunks were tested after this injection. In these tests we were able to show a temporary but definite improvement in the functional state of these same nerves. Lability grew and the amplitude of the action currents increased. Figure 3 represents the oscillogram of the biocurrents registered from the parasympathetic cervical nerves of a cat which, 8 days previously, had been subjected to a partial pancreatectomy. When the stimulation frequencies rose from 100 to 125 cps, there could be observed a sharp decrease in the amplitude of the action currents (A). Further increase in the frequency to 150 cps resulted in inhibition of the electrical activity of the nerve (B) (the right side of the film shows only eddy currents). One hour after injecting the acetylcholine (C) the amplitude of the action currents grew with stimulation at a frequency of 100 cps. Increasing the frequencies to 125 (D) and 150 cps (E), while decreasing the amplitude of the action currents, did so to a much lesser degree than seen before the introduction of the acetylcholine.

The conclusion can then be drawn that removal of the major portion of the pancreas leads to definite functional alterations within the nerve trunks. There is observed an increase in the length of both the absolute and relative refractory phases and a shortening (in the majority of instances an absence) of the period of reinforcement. The threshold of reactivity rises while basic lability levels decrease. All this is consistent with a situation in which action currents leave residual effects for a longer time, which is another way of saying that restorative processes have become prolonged. Systematic introduction of acetylcholine into the operated animals compensates the indicated abnormalities. It is instructive to record the fact that introduction of the acetylcholine in to the operated animals improves the functional state of the nerve trunks only as long as they maintain their anatomical ties with the nerve cells. This question has been explored more thoroughly by Kh. S. Khamitov [7] who showed that in the operated animals nerve irritability could be restored to normal either by injecting the acetylcholine intraveneously as long as the nerve trunks maintained their connections with their nerve cells or by injecting the chemical mediator directly into the nerve trunk itself.

Our experiments permit us the conclusion that acetylcholine participates in the functional regulation of the processes determining nerve fiber activities. It seems probable that acetylcholine is one of the components of the current of excitation and that it facilitates the functional transformations of the nervous tissue in such a fashion that conditions for better transmission of the nerve impulses are created, the excitability and level of lability of the nerve conductor being raised.

SUMMARY

The most important indices of the functional condition of the autonomic nerve trunks, i.e., excitability, lability and refractivity, were investigated in normal and in operated animals. Removal of a large part of the pancreas in cats brings about a fall in the level of lability and increased refractivity of the nerve trunks. Acetylcholine abolishes these disturbances.

It is suggested that acetylcholine plays a significant role as a factor promoting change of the functional condition of the nerve tissue, as well as in preparation of the best conditions for transmission of nerve impulses by way of the nerve trunk.

LITERATURE CITED

[1] Babskii, E. B., Fiziol. Zhur. XXIV, No. 3, 536-545, 1938.

[2] Babskii, E. B., and Kovyrev, I. G. Biull. Eksptl. Biol. i Med. XVII, No. 6, 30-33, 1944.

[3] Babskii, E. B., Proceedings of 7th All-Union Congress of Physiologists, Biochemists and Pharmacologists,* I, 319-322, 1947.

[4] Zefirov, L. N. and Kibiakov, A. V., Fiziol. Zhur. XL, No. 2, 183-190, 1954.

[5] Kibiakov, A. V. and Uzbekov, A. A., Biull. Eksptl. Biol. i Med. XXIX, No. 3, 202-205, 1950.

[6] Kovtun, S. D., Nauch. zap. Kiev un-ta., Kiev, XII, No. 8, 193-209, 1953.

[7] Khamitov, Kh. S., Some Mechanisms of the Nervous Regulation of Smooth Muscle,* Candidate's Dissertation, Kazan, 1953.

[8] Ukhtomskii, Fiziol. Zhur., XXV, No. 6, 767-778, 1938.

[9] Bronk, D. W., Journ. Neurophysiol. 1939, Vol. 2, pp. 380-401.

[10] Calabro, Q., Riv. Biol. 1933, Vol. 15, pp. 229-320.

[11] Cowan, S., Journ. Physiol. 1938, Vol. 93, pp. 215-262.

[12] Lorente de No R., Journ. Comp. Cell. Physiol. 1944, Vol. 24, pp. 85-97.

[13] Nachmansohn, D., Journ. Neurophysiol. 1941, Vol. 4, pp. 348-361.

* In Russian.

DIRECTION AND LINEAR VELOCITY OF FLOW OF CEREBROSPINAL FLUID IN THE SPINAL SUBARACHNOID SPACE

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(Received January 12, 1957. Presented by P. S. Kupalov, Active Member Acad. Med. Sci. USSR)

The direction of flow of cerebrospinal fluid in the spinal subarachnoid space has been the subject of controversy since the end of the last century.

Thus Falkenheim and Naunin [18], Becher [12-14], S. N. Sharavskii [10], Sachs, Wilkins and Sams [22], Hassin [19], I. A. Alov [1], and others deny that there is any flow of cerebrospinal fluod. Jacob [21], Strecker [23], A. D. Speranskii [7], Eichler, Linder and Schmeiser [16, 17], D. A. Shamburov [9], and others maintain that it flows in an upward direction, while Hill [20], Weigeldt [25], N. D. Khodiakov [8], L. L. Papadato [6], D. A. Zhdanov [3], Tubiana, Benda and Constans [24], Becker [15], and others believe that it flows downwards. Finally, Ahrens [11], G. F. Ivanov [4], and others consider that it may flow in both directions, upward and downward.

This conflict of views may to a certain extent be ascribed to the imperfections of the methods applied. Most of the investigators used various dyes, and based their conclusions as to direction of flow on the movements of the dyes from the locus of introduction. Those authors who introduced the dye into the lumbar subarachnoid space, and who then found it in the cranial spaces, concluded that the direction of flow was cranial.

Authors who introduced the dye into the suboccipital cistern, and who later found it to be present in the lower parts of the spinal canal, concluded that the fluid flows caudally. Finally, the authors who injected dyes into both the suboccipital and the lumbar subarachnoid spaces concluded that either the fluid moves in both directions, or that it does not flow at all. These studies did not take into consideration that respiratory and pulse rhythms are of fundamental importance to the dynamics of cerebrospinal fluid. These factors bring about a continuous mixing of cerebrospinal fluid, and are responsible for so-called turbulent diffusion of dyes [2].

Taking into account these features of the hydrodynamics of the cerebrospinal fluid, we came to the conclusion that, in order to be able to determine the direction of flow as shown by movements of diffusible substances, it is necessary to compare the concentrations of such substances above and below the point of introduction. The direction in which the greater part of the indicator substance moves would be that of flow of cerebrospinal fluid, since in that direction the rate of movement of indicator would represent the sum of that of progressive flow of cerebrospinal fluid and of diffusion of indicator substance.

Radioactive indicators are the most convenient for quantitative measurements.

EXPERIMENTAL METHODS

The experiments were performed on cats, under urethane narcosis (1 g per kg body weight). The animals were kept warm by means of electric heaters during the experiments. We performed 8 experiments, including 3 control ones. Radiophosphorus (disodium phosphate containing P^{32}) was taken as the radioactive indicator. We chose this tracer because of the important role played by phosphorus in many of the metabolic processes of the body, so that it is the most physiological indicator. Apart from this consideration, radiophosphorus was the most readily available. We used radiocalcium ($Ca^{42}Cl_2$) in one of our control experiments. We introduced P^{32} at a dosage of 1,000,000 i.p.m. per kg body weight, in 0.02 ml of physiological saline. Suboccipital and lumbar

puncture were performed with the animal lying on its side in a horizontal position, using a fine needle, with a clamp and a fitting for a micropipet. A hole was dripped through the lamina of a vertebra midway between the two punctures (at the level of T-6), and the solution of radioactive indicator was introduced from a micropipet, through a fine straight or bent needle, into the subarachnoid space, taking 10 seconds for the injection, and keeping the pressure as low as possible. The point of the needle was always directed cranially during the injection. After removing the needle, the opening in the vertebral lamina was closedhermetically with a plexiglass plug.

Samples of cerebrospinal fluid (0.01 ml) were taken simultaneously by means of micropipets from the suboccipital and lumbar subarachnoid spaces, after 5, 30, 60, 120, 180, 240, 300, 360, and 480 minutes, and were transferred to aluminum dishes with a filter paper disc attached to the bottom, for ensuring uniform distribution of the sample.

In addition, we measured the activity of the dura mater, caudial and cranial to the point of injection, along the whole length of the vertebral canal. We chose to measure the activity of the dura mater, rather than of the pia mater or the arachnoid, or of medullary tissue, because it is greater than the others (our own observations, and those of N. A. Maiorova [5]). After each experiment the animals were killed by exsanguination. The cavity of the vertebral canal was opened up section by section. We first exposed the spinal cord at the level of introduction of the indicator, and applied a ligature, and then repeated the operation at the level of the first cervical vertebra, in order to prevent any displacement of cerebrospinal fluid during the subsequent trepanation. The next stage was to open up the canal from the direction of lesser to greater activity. During the opening up of the vertebral canal we discarded the instruments after each few manipulations, and cleaned them thoroughly with 10% caustic alkali and copious amounts of water, so as to eliminate possible contamination. After opening up the vertebral canal we took specimens of dura, which we washed withphysiologicalsaline for a fixed time, dried with filter paper to the same humidity, weighed on a torsion balance, and distributed 25-mg portions evenly on sampling dishes.

The activity of the dried specimens was measured by means of an end-window counter in a lead chamber, using a Type B equipment. The results were expressed as i.p.m. per 100 mg of fresh tissue or per 0.1 ml of cerebrospinal fluid, applying appropriate background corrections. The relative error of measurement did not exceed 3-4%. Background radiation remained at a constant level during the experiments (about 12-15 i.p.m.).

EXPERIMENTAL RESULTS

After introduction of radiophosphorus into the subarachnoid space at the level of T-6, the activities of cerebrospinal fluid taken from the lumbar and suboccipital subarachnoid spaces were found to vary in accordance with the typical curves of Fig. 1. Activity was zero in the suboccipital cistern after 5 minutes, and had risen after 120-180 minutes to 240-372 i.p.m., falling to 24-44 i.p.m. by the 8th hour. The cerebrospinal fluid of the lumbar subarachnoid space showed a different picture. Activities of the order of 160-840 i.p.m. were found at the end of 5 minutes. The activity rose for 60-120 minutes, to a maximum of 1860-3250 i.p.m. It then fell, amounting by the 4th-8th hour to 990-1020 i.p.m. (Table 1).

We found in the same experiments that the activity of the dura was not uniform along the length of the vertebral canal; it was maximum at the level of the injection, amounting to 99,000 i.p.m. after 4 hours, and to 8,000-13,000 i.p.m. after 8 hours. Parts of the dura cranial to T-6 showed less activity than did those taken caudal to T-6.

The differences in activity are fully significant, since they are many times greater than the mean experimental error. Although the curves vary markedly from individual to individual, they all present the same general picture (Table 2).

The question arises whether these results might not be a consequence of aspiration of cerebrospinal fluid, with production of an artificially directed flow toward the spinal subarachnoid space.

In order to refute this objection we performed 3 control experiments, in which we injected radiophosphorus solution (radiocalcium in Experiment 59), but did not withdraw cerebrospinal fluid from the suboccipital and lumbar subarachnoid spaces. Subsequent measurement of dural activity showed excess activity of caudally located specimens 4 hours after injection (see Table 2, Experiments 52, 56, and 59).

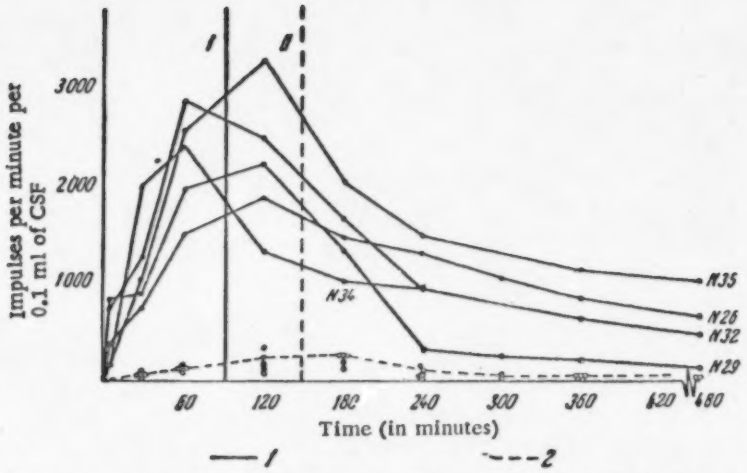

Fig. 1. Activity curves of cerebrospinal fluid taken from the suboccipital and lumbar subarachnoid spaces after subarachnoid injection of radiophosphorus at the level of T-6.
Explanation of curves: 1) activity in the lumbar space; 2) activity in the suboccipital cistern; first vertical line (I) average time needed for development of maximum activity in the lumbar space; second vertical line (II), in the suboccipital cistern.

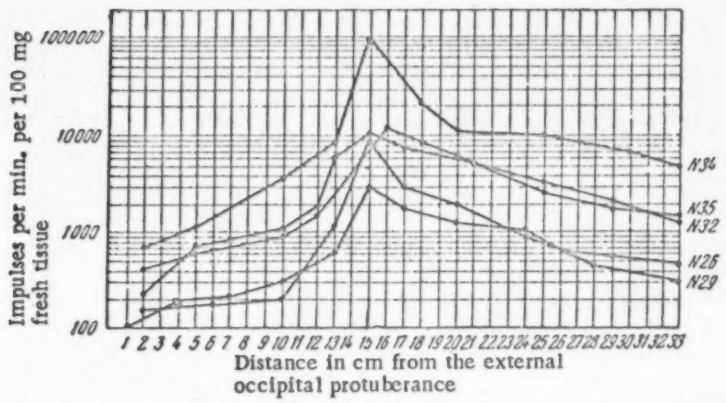

Fig. 2. Activity of the dura mater at different levels of the spinal cord caudal and cranial to the point of injection of radioactive phosphorus.
Explanation of curves: ordinates – activity in impulses per minute per 100 mg of fresh tissue, on a logarithmic scale; abscissae – distance in cm from the external occipital protuberance.

Apart from this, it is noteworthy that the first sample withdrawn from the lumbar space showed a higher activity than did that taken at the same time from the suboccipital cistern.

Our results are in agreement with the view that the flow of cerebrospinal fluid in the spinal subarachnoid space is in a caudal direction, where channels are located for its outflow into the vascular and lymphatic beds. This flow is so slow as to be of the same order of magnitude as is the process of diffusion of radiophosphorus in

TABLE 1

Relative Activity in the Suboccipital and Lumbar Cisterns after Introduction of Radiophosphorus at the Level of T-6

No. of experiment \ Time in minutes	Activity cerebrospinal fluid																
	in the lumbar cistern									in the suboccipital cistern							
	5	30	60	120	180	240	300	360	480	5	30	60	120	180	240	300	360	450
26	350	740	1 510	1 860	1 450	1 310	1 070	850	650	0	40	84	128	280	92	60	52	24
29	840	940	1 990	2 200	1 390	350	280	240	160	0	12	132	164	264	52	48	40	32
32	720	1 260	2 840	2 480	1 670	950	—	640	510	0	16	136	240	148	104	—	36	28
34	170	2 000	2 400	1 300	1 020	990	—	—	—	0	72	180	372	204	184	—	—	—
35	160	1 020	2 560	3 250	2 000	1 500	—	1 150	1 020	0	24	86	136	284	76	—	60	44

TABLE 2

Relative Activity of the Dura Mater at Different Levels of the Spinal Cord after Subarachnoid Introduction of Radiophosphorus at the Level of T-6 (impulses per min. per 100 mg fresh tissue)

No. of experiment	Duration of experiment \ Distance cm from ext. occipital protuberance	1	2—4	5—7	8—10	11—12	13	14	15	16—17	18—19	20—23	23—24	25—28	29—30	32—33
26	8 hours	100	190	212	300	—	632	—	3 020	1864	—	1 220	1 140	662	572	500
29	8 hours	—	152	184	212	—	1 180	—	8 132	3 000	—	2 104	990	460	—	342
32	8 hours	—	412	640	896	1 580	—	4 152	—	13 420	8 900	6 314	—	3 940	2 120	1 350
34	4 hours	—	716	1 016	3 900	—	8 792	—	99 670	—	22 200	11 750	—	10 060	6 200	4 900
35	8 hours	—	236	752	1 020	1 940	6 825	—	10 150	7 450	—	5 800	—	2 932	1 852	1 600
52	4 hours	—	480	540	756	—	6 542	—	19 620	990	—	8 000	—	4 200	3 120	1 232
56	4 hours	—	284	580	684	—	742	—	5000	17 800	—	6 592	—	3 264	2 000	1 024
59	4 hours	212	—	236	676	912	—	1 184	2756	—	—	2 480	—	2 016	1 968	—

the cerebrospinal fluid, under the given conditions, since radiophosphorus spreads not only in the direction of flow of cerebrospinal fluid, but also in the opposite direction. This is why we found some activity in the cerebrospinal fluid of the suboccipital cistern and in the dura of the cervical spinal cord.

It is possible from our data to make an approximate calculation of the rate of flow of cerebrospinal fluid in the spinal subarachnoid space. If we take the distance from the point of injection to the sites of lumbar and suboccipital puncture as being equal to 15 cm, and the time needed to attain maximum activity of the cerebrospinal fluid as being 96 minutes in the lumbar space, and 156 minutes in the suboccipital cistern, we calculate the linear rate of flow in a caudal direction to be 1.56 mm per minute, and in a cranial direction 0.961 mm per minute. These are not, however, the real values, as they need to be corrected for diffusion. An analysis of the origin of the flow rates in a caudal and a cranial direction permits of an evaluation of this correction.

The observed rate of flow in the caudal direction ($V_{obs.\ caud.}$) is the sum of two rates: diffusion ($V_{diff.}$), and progressive displacement of cerebrospinal fluid, due to its continual resorption from the spinal subarachnoid space ($V_{CSF\ real}$). This relation may be expressed by the following equation:

$$V_{osb.\ caud.} = V_{CSF\ real} + V_{diff.} \qquad (1)$$

The observed flow rate in the cranial direction ($V_{obs.\ cran.}$) is the difference between the rate of diffusion and the actual rate of flow of cerebrospinal fluid downwards:

$$V_{obs.\ cran.} = V_{diff.} - V_{CSF\ real} \qquad (2)$$

We thus have two equations with two unknowns. Substituting the value of $V_{diff.}$ given by Equation (2) in Equation (1) we obtain:

$$V_{CSF\ real} = \frac{V_{obs.\ caud.} - V_{obs.\ cran.}}{2} = \frac{1.56 - 0.961}{2} = 0.299 \text{ mm/min.}$$

Our findings as to the direction of flow of cerebrospinal fluid are in agreement with the observations of Becker and of Tubiani, Benda and Constans. Becker found that a dye (Indian blue) introduced into the spinal subarachnoid space of rabbits was displaced after 2-3 hours 2-3 cm further in the caudal than in the cranial direction, and that the intensity of coloration seemed greater in the lower than in the upper subarachnoid spaces.

According to Tubiani, Benda and Constans, radioactive sodium present in the spinal subarachnoid space of patients under their care diffused faster from above down than from below up.

The practical conclusion might be drawn from our experimental data and from those given in the literature that it is not advantageous to introduce drugs used for the treatment of inflammatory conditions of the brain and meninges into the lumbar subarachnoid space.

Our experimental data are evidence that cerebrospinal fluid flows in a caudal direction through the spinal subarachnoid space. In cats, the mean linear rate of flow in the caudal direction amounts to 0.3 mm/min. Diffusion plays a substantial part in the distribution of substances in the cerebrospinal fluid in the spinal subarachnoid space.

SUMMARY

The direction and velocity of motion of the cerebrospinal fluid in the spinal subarachnoid space was studied in experiments on cats under urethane anesthesia with the aid of radioactive phosphorus (P^{32}). The latter was introduced into the spinal subarachnoid space at the level of the 6th thoracic vertebra. The activity of cerebrospinal fluid was investigated at definite intervals in the suboccipital and lumbar cisterns and it was revealed that it moved in a caudal direction with the velocity of 0.3 mm per min.

Diffusion plays a significant part in distribution of radioactive substances in the spinal subarachnoid space.

LITERATURE CITED

[1] I. A. Alov, Voprosy Neirokhirurg, 13, No. 5, 28-34 (1949).

[2] M. A. Velikanov, Dynamics of Flow in Ducts,* Vol. 1, Moscow 1954.

[3] D. A. Zhdanov, Klin. Med. 26, No. 7, 15-22 (1948).

[4] G. F. Ivanov, Trudy Nauch.-Issled. Inst. Ukha, Gorla i Nosa, 6, 309-314, Moscow 1955.

[5] N. A. Maiorova, "Experimental Study of Absorption of Erythrocytes from the Interthecal Spaces"* (Thesis), Moscow, 1955.

[6] L. L. Papadato, Transactions Nervous Diseases Clinic, Odessa Med. Inst. 1, 97-180 (1929).

[7] A. D. Speranskii, Elements for the Construction of a Theory of Medicine,* Moscow, 1935.

[8] N. D. Khodiakov, Vestnik Oto-rino-laringol., No. 3-4, 359-363 (1927).

[9] D. A. Shamburov, Cerebrospinal Fluid,* Moscow 1954.

[10] S. N. Sharavskii, Cerebrospinal Fluid,* Kiev 1928.

[11] H. Ahrens, Ztschr. ges. Neurol. u. Psych. 1913, Vol. 50, No. 5, pp. 578-593.

[12] E. Becher, Zbl. f. inn. Med. 1920, No. 37, pp. 633-651.

[13] Ibid, Münch. med. Wschr. 1921, Vol. 68, No. 27, pp. 1839-1840.

[14] Ibid, Mitt. Grenzgeb. Med. and Chir. 1922, Vol. 35, pp. 366-388.

[15] H. Becker, Nervenarzt, 1952, Vol. 23, No. 4, pp. 146-148.

[16] O. Eichler, F. Linder and K. Schmeiser, Klin. Wschr. 1951, Vol. 29, No. 1, pp. 9-12.

[17] O. Eichler, F. Linder and K. Schmeiser, Klin. Wschr. 1952, Vol. 29, No. 41-42, pp. 707-708.

[18] H. Falkenheim and B. Naunin, Arch. exp. Path. and Pharm. 1887, Vol. 22, pp. 261-305.

[19] G. B. Hassin, Journ. Amer. Med. Assoc. 1933, Vol. 101, No. 11, pp. 921-923.

[20] L. Hill, The Physiology and Pathology of the Cerebral Circulation. London, 1896.

[21] P. Jacob, Dtsch. med. Wschr. 1900, No. 3, pp. 46-48.

[22] E. Sachs, H. Wilkins and F. S. Sams, Arch. Neurol. a. Psychol. 1930, Vol. 23, No. 1, pp. 130-152.

[23] H. Strecker, Münch. med. Wschr. 1922, Vol. 69, No. 50, pp. 1726-1729.

[24] M. Tubiana, Ph. Benda and J. Constans, J. Rev. Neurol. 1951, Vol. 85, pp. 17-35.

[25] W. Weigeldt, Studien zur Physiologie und Pathologie der Liquor cerebrospinalis, Jena, 1923.

* In Russian.

APPLICATION OF STATIC MUSCULAR CONTRACTION TO THE ANALYSIS OF THE MECHANISM OF VOMITING

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According to a number of authors [1, 6, 9] many of the functions of the organism are inhibited when isometric concentrations of muscles are initiated. We thought it would be of interest to apply these features of static work to the analysis of the mechanism of vomiting. It is known that a whole complex of reactions, both of a somatic and of an autononnic nature, is involved in the act of vomiting. We examined the inhibitory effect of static work on the vomiting reaction.

EXPERIMENTAL METHODS

The experiments were performed on dogs with Basov gastric fistulae and with a Thiry -Vella fistula of the jejunum. Vomiting was induced in two ways: reflexly, by stimulating gastric receptors, and by direct action on the vomiting center by means of apomorphine, 0.1% solutions of which were prepared before the experiments, and were injected subcutaneously in small doses (0.03-0.05 mg/kg). At such dosage levels, apomorphine caused vomiting within 8-15 minutes. The experiments were performed at 3-day intervals, with a break of two days between them (under such conditions habituation of apomorphine did not take place [4]). Reflex induction of vomiting was achieved by introducing 500 ml of 15% potassium chloride through a gastric fistula, during 40 seconds. Vomiting ensued (1 or 2 attacks) after 5-7 minutes. In such experiments the potassium chloride solution acts reflexly on the vomiting center [2]. We registered the respiration of the animals, as well as gastric or small intestine contractions and the pulse rate. We evaluated the reaction of the abdominal muscles and diaphragm from the nature of the change in intra-abdominal pressure at the moment of vomiting, which affected the gastric contraction tracings. We also recorded the rate of onset of premonitory signs (licking of lips, salivation, lassitude).

The muscle loading consisted of fastening a sand-bag, half the weight of the animal, to its back. The load was maintained until the dog showed clear signs of fatigue. Vomiting was induced while it was still carrying the load. Experiments involving loading were alternated with control experiments. In all, we performed 212 experiments on 6 dogs, 65 of the experiments involving load-bearing.

RESULTS OF EXPERIMENTS

Evaluation of the effects of static work on vomiting showed that, in general, such tensions inhibited vomiting. The strength of the inhibitory effect depended on a number of factors. If loading was not applied initially, but only after a number of days, during which vomiting was induced 7-10 times, only very slight inhibitory effects were observed. If the load was applied at once, without previous induction of vomiting, its inhibitory effect appeared sooner, and was more considerable. The small inhibitory effect observed in the former conditions can be ascribed to elaboration of a stable "vomiting" stereotype, which was difficult to overcome.

The inhibition of vomiting produced in the first experiments with static loading became strengthened with time, to such an extent that double doses of apomorphine became ineffective. After a number of experiments involving load-bearing we found that inhibition of vomiting was produced merely by the experimental environment, i.e., by a conditioned reflex mechanism. In the control experiments (not under working conditions)

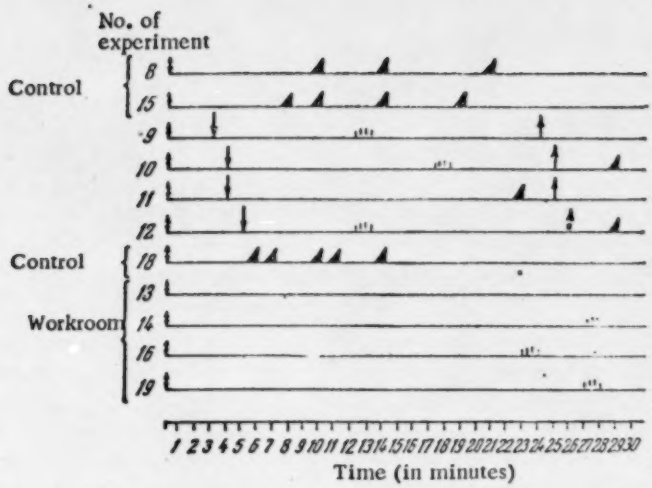

Fig. 1. Effect of static loading and of the working environment on the vomiting reaction.
Explanation of symbols: ◢ vomiting; |||| dyspnea; ⇕ injection of apomorphine; ↓ application of load; ↑ removal of load.

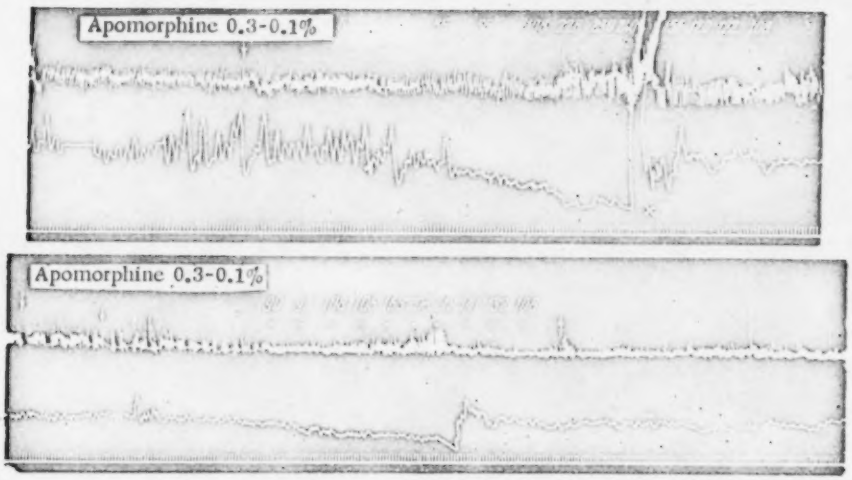

Fig. 2. Effect of static loading on the vomiting mechanism.
Explanation of tracings (from above down): respiration, stomach contractions, time marker (5 seconds), ↓ application of load, ↑ removal of load. Upper figures – pulse rates; × – abrupt rises in the gastric contraction tracing, due to contraction of abdominal muscles. Under conditions of load-bearing (lower kymogram) the reaction due to the abdominal muscles is inhibited, but the reactions due to the stomach, to respiration, and to the cardiovascular system are retained.

vomiting took place with the usual intensity (Figure 1). In many of the experiments conditioned reflex inhibition of vomiting exceeded unconditioned reflex inhibition. We thus see that the effect of the cerebral cortex, achieved in the given case according to the principle of the dynamic stereotype, is an important factor in the development of resistance of the organism to the action of emetics. Our findings agree in many respects with those of A. O. Dolin [3], who used different emetics.

Inhibition of reflex vomiting due to the action of potassium chloride proceeded in the same order, but was achieved somewhat faster and more readily. Here, too, we observed conditioned reflex inhibition of the vomiting reaction.

A detailed analysis of the effect of static loading on vomiting showed that this factor does not inhibit the different components of the vomiting reaction to the same extent. The soonest and the most readily inhibited component was that due to contraction of the skeletal muscles of the abdominal press (Fig. 2), which in effect prevented the onset of vomiting. The autonomic components, such as "vomitory" contractions of the stomach, salivation, dyspnea, and tachycardia, were much less affected. These reactions were retained in most of the experiments involving load-bearing. The complex of autonomic reactions encountered in vomiting due to potassium chloride was more profoundly inhibited than with apomorphine. Here the reaction from the respiratory center was missing, as well as that due to contraction of abdominal muscles.

Since static work inhibited only one of the components of the vomiting reaction, leaving the remaining components intact, we proceeded to study the extent of suppression of vomiting under conditions of more profound inhibition of the central nervous system, viz., under narcosis. Our experiments, performed on 10 dogs, showed that narcosis had a more powerful inhibiting effect on vomiting due to injection of apomorphine, in doses of 0.15-2 mg per kg, than did static loading. Not all the autonomic components were abolished here, however. Apart from inhibition of the abdominal muscle component, we observed inhibition of gastric contractions and of salivation, but the blood pressure and respiration components were more persistent (Fig. 3).

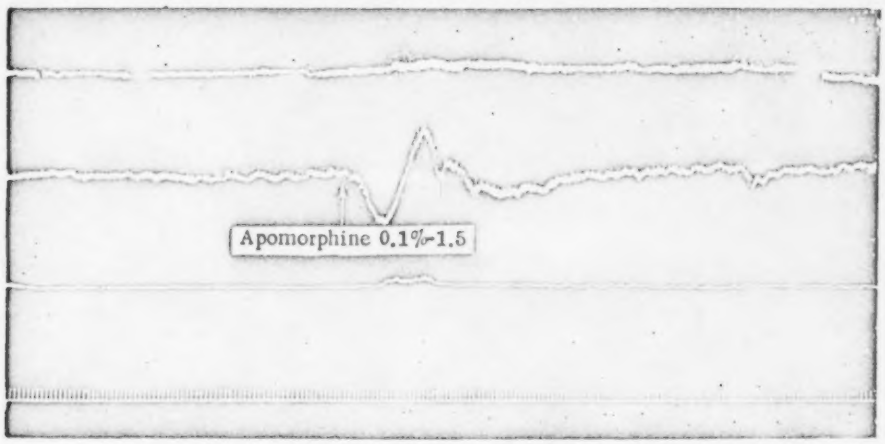

Fig. 3. Reaction of anesthetized dogs (chloroform-ether mixture) to apomorphine. Explanation of tracings (from above down): respiration, blood pressure, gastric contractions, time marker (5 seconds). Reactions from the stomach and from the abdominal muscles are abolished. Reactions from blood pressure and respiratory components are retained.

Our observations showed that the individual components of the vomiting mechanism enter into operation in a fixed sequence. As is evident from Fig. 2, the first reaction to introduction of apomorphine is from the stomach, in the form of a gradual fall in its tonus. This is soon followed by reactions from other systems. This permits one to assume that the vomiting mechanism begins with excitation of the motor centers of the gastrointestinal tract. This excitation increases in intensity, and radiates to a number of other centers: the salivation center (the dog starts licking its lips), the cardiovascular center, the respiratory center, and then to motor centers of the skeletal muscles. As appears from Fig. 2 (lower kymogram), stimulation of the motor centers of the gastrointestinal tract bears a clearly-expressed inductive-reciprocal nature: prolonged and profound inhibition of stomach contractions is followed at the moment of expected vomiting by violent contractions. The effect here is one of sequential inversion of stimulatory processes in the autonomic centers: stimulation of sympathetic centers is followed by their inhibition, and the previously reciprocally inhibited parasympathetic centers now enter into

a state of powerful stimulation. The stimulation of these centers is the more powerful the more prolonged the phase of inhibition.

We often observed in our experiments that the somatic muscles did not immediately enter into the vomiting mechanism, i.e., that there was a time lag in the passage of stimulation from the autonomic to the somatic centers. A time lag of this sort was first described by Sherrington [11], although not for the vomiting reaction. A hindrance to passage of stimulation from autonomic to somatic centers has also been noted by V. N. Chernigovskii and O. S. Merkulova [8].

From the above considerations we conclude that the realization of the act of vomiting is made possible by the considerable intensification of stimulation of autonomic centers, mediated by inductive-reciprocal relations. The stimulatory process, reinforced in this way, is enabled to overcome the impedance, and thereby to pass over from the autonomic to the somatic centers.

As is evident from the above, we do not consider that the existence of special formations corresponding to the concept of a vomitory center is essential. It follows that we do not admit the existence of a single center regulating the motor activity of the gastrointestinal tract, moderate stimulation of which assures normal working of the stomach, while stronger stimulation causes vomiting. This view coincides with that of A. I. Mordovtsev [5].

In accordance with I. P. Pavlov's teaching regarding centers [7], we should take a much broader view of the vomiting center, in its topographic aspect. We believe that the mechanisms regulating vomiting are located not only in the medulla oblongata, but also in other parts of the central nervous system. Thus the observation that the application of static loading results in the first place in the abolition of the press effect of the abdominal muscles and the diaphragm justifies the assumption of a special location of somatic muscle centers participating in the act of vomiting. This assumption agrees with the findings of Openchowski [10], who showed that the somatic component of the vomiting mechanism may be abolished by destroying certain subcortical formations of the brain. We think that the meaning which is now attached to the concept of the vomiting center should be restricted to the afferent limb of the reflex arc only, represented in the medulla oblongata both by sensory cells and by special chemoceptors [12]. As Pavlov showed, it is this receptor part which is the most important for any center. The efferent part is to be regarded as being purely executive in function; one and the same efferent apparatus may be applied for various purposes.

On the basis of the foregoing considerations regarding the regulation of the mechanism of vomiting, we consider its inhibition by application of static work to be the result of negative induction from certain definite cells of the motor analysor, in which a concentrated focus of excitation is formed under the influence of impulses from proprioceptors arising at the moment of static loading. It is evident that inductive inhibition will in the first place affect somatic muscle centers. The autonomic centers will be less powerfully inhibited. Although "vomitory" stimulation could arise in them, it would not be powerful enough to reach the somatic muscle centers and to stimulate them to activity.

SUMMARY

Static muscular work which consisted in holding a load on the back inhibited the act of vomiting which was induced by apomorphine or by stimulation of the stomach receptors by a solution of potassium chloride. The act of vomiting was inhibited by placing the dog under conditions in which the experiment was formerly conducted (following several experiments with the load, i.e., by way of conditioned reflex). Various components of the act of vomiting were inhibited differently. The mildest depression was found in the activity of somatic muscles (abdominal press). The autonomic component of the act of vomiting (secretion of saliva, dyspnea, stomach contraction, increased rate of cardiac contractions) was only slightly inhibited. With increase of inhibition (anesthesia) the reactions of blood pressure and respiration were found to be the most stable components out of the whole autonomic complex of vomiting reactions. A definite sequence of introduction of various components of the act of vomiting was established in experiments without the load. Several new suggestions are made concerning the regulation of the act of vomiting.

LITERATURE CITED

[1] I. S. Aleksandrov, Arkh. Biol. Nauk SSSR, 32, No. 5-6, 364-374 (1932).

[2] I. A. Bulygin, Biull. Ekspti. Biol. i Med. 29, No. 6, 95-99 (1950).

[3] A. O. Dolin, in: I. P. Pavlov's Teaching in Theoretical and Practical Medicine,* 409-447, Moscow 1953.

[4] F. G. Dubinin, Arkh. Biol. Nauk SSSR 42, No. 3, 16-19 (1936).

[5] A. I. Mordovtsev, Biull. Ekspti. Biol. i Med. 36, No. 9, 19-21 (1953).

[6] V. A. Novi, Transactions VIII All-Union Congress of Physiol., Biochem. and Pharmacol. 451-453, Kiev, 1955.

[7] I. P. Pavlov, Twenty Years of Experience of Objective Study of Higher Nervous Activity in the Behavior of Animals,* p. 95, Moscow 1951.

[8] V. N. Chernigovskii and O. S. Merkulova, Biull. Ekspti. Biol. i Med. 29, No. 1, 43-47 (1950).

[9] R. A. Shabunin, "Effect of Static Exertion on Unconditioned and Conditioned Vascular Reflexes in the Human" (Thesis) Sverdlovsk 1955.*

[10] Openchowski, Arch. f. Anat. und Physiol. 1889, H. 1-2, pp. 549-556.

[11] C. Sherrington, The Integrative Action of the Nervous System, Cambridge, 1906.

[12] S. G. Wang and H. L. Borison, Gastroenterology, 1952, Vol. 22, pp. 1-12.

* In Russian.

REFLEX REGULATION OF CAPACITY FOR WORK

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The possibility of raising the capacity of a muscle to perform work, by applying various external factors, has been demonstrated by V. O. Bugoslavskii [1], N. I. Konopasevich [5], and I. M. Sechenov [9], and has been confirmed by the work of numerous Russian and foreign authors. D. I. Shatenshtein and E. N. Iordanskaia [11] not only showed that the working capacity of the right arm could be raised by using the left arm, but also revealed that dependence of this effect on the strength of the additional stimulation and on the time intervals between stimulations. Soviet workers have also shown that it is possible to raise working capacity by means of conditioned reflex action, including the use of verbal signals [2, 3, 6, 7, 10]. Many aspects of reflex regulation of working capacity, of importance to the physiology of work and sport, are still insufficiently elucidated.

The present paper is devoted to the study of the effects of auditory stimuli and of skin temperature and motor analysors on the working capacity of the fatigued person.

EXPERIMENTAL METHODS AND RESULTS

The experimental subjects were put to work on an ergograph; by flexing the right arm at the elbow they raised a lever with a load of 10-14 kg 30 times a minute. The weight of the load raised by the right arm was determined for each individual after a prolonged period of training. Further observations were carried out with a load such that marked lowering of working capacity took place within 8-10 minutes of work. Supplementary stimulation of analysors was applied with a $1\frac{1}{2}$-2-fold reduction of the height to which the load was raised. Only one supplementary stimulus was applied during an experiment. The auditory analysor was stimulated by means of a bell, loudness 50 decibels, and the skin temperature analysor by application of hot (40-44°) or cold (0, −1, −4, −7°) bodies to the skin over the biceps muscle of the arm which was not working.

Supplementary stimulation of the motor analysor was achieved by starting work with the left, previously inactive, arm. The movements of the left arm took place at the same rate as the right, but against a much smaller load (1-2 kg), or without a load.

Our observations showed that sounding the auditory stimulus caused a marked rise in working capacity. Daily repetition of the experiments showed that this effect was very transient (Fig. 1); for most subjects (7 out of 8) it disappeared after 2-4 repetitions.

Heat stimulation of the skin temperature analysor raised the amount of work performed by all the subjects, while cold stimulation produced this effect in two of three subjects. Repetition of the experiment, with both cold and heat stimulation, also led to a rapid extinction of the effect (after 2-3 repetitions)(Fig. 2). Only in one of the subjects (G.) did we find a relatively slow disappearance of the effect, both for auditory and temperature stimulation.

It is of interest that the amount of work performed was raised by the action of the verbal stimuli: "Cold" or "Heat."

Supplementary stimulation of the motor analysor, by starting work with the left arm when the right was showing signs of fatigue, raised the amount of work performed by the right arm (Fig. 3, A, B). During the first

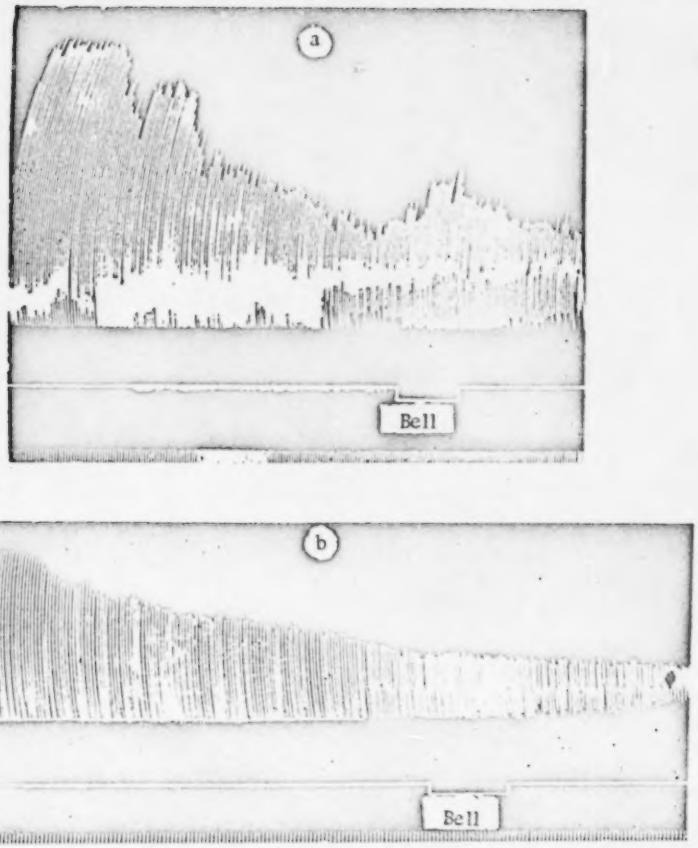

Fig. 1. Changes in working capacity due to stimulation of the auditory analysor. Explanation of tracings (from above down): ergogram, stimulation signal, time marker (1 second), a,b) data from different experiments.

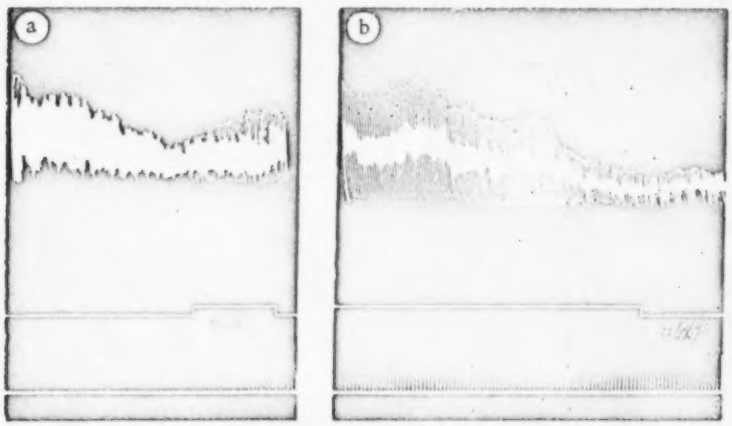

Fig. 2. Changes in the working capacity of the arm in response to stimulation of the temperature analysor.
Explanation of curves (from above down): ergogram, stimulation signal, time marker (1 second); a and b) data from different experiments.

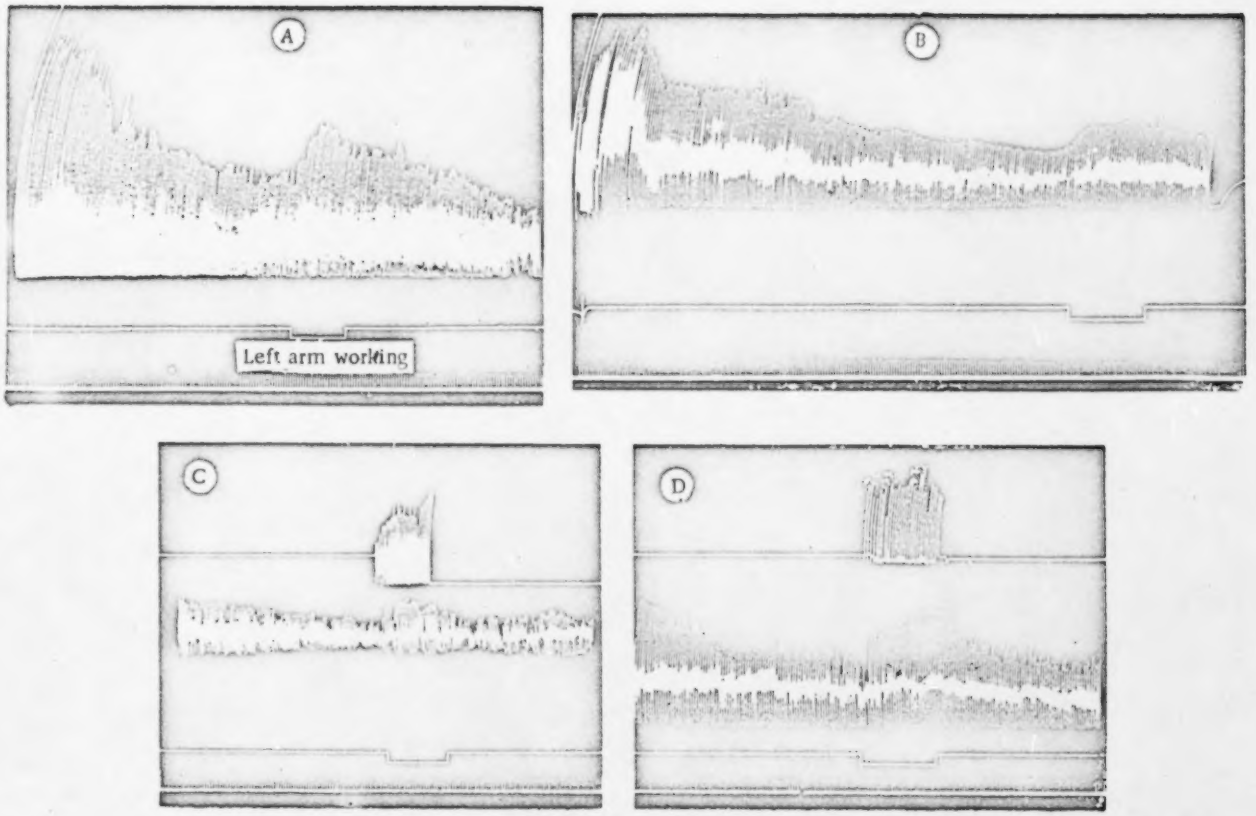

Fig. 3. Changes in working capacity due to the effect of supplementary stimulation of the motor analysor.
A and B with active, and C and D with passive movements of the left arm.
Explanation of tracings (from above down): for A and B, ergogram for the right arm, signal showing when the left arm began work, time marker (1 second); for C and D, recording of passive movements of the left arm, ergogram for the right arm, signal showing stimulation (passive movements of the left arm), time marker (1 second).

experiments this effect did not differ from that observed with stimulation of the auditory analysor, but a significant difference appeared as the experiments were continued: numerous repetitions (up to 30) did not reveal any tendency toward weakening of the effect.

In our next experiments we set in motion a device which caused passive movements of the left arm, at a time when the right arm began to show signs of fatigue. We found that passive movements of the left arm also raised the work performance of the right arm. The magnitude of this effect did not diminish with repetition of the experiment (Fig. 3, C, D).

In our further experiments we attempted to elucidate the effect of supplementary stimuli on the reactions which ensure the achievement of muscular work.

We performed experiments in which we studied the effects on gaseous metabolism and pulmonary ventilation of stimulation of the auditory analysor, and of the motor analysor by means of passive movements.

TABLE 1

Changes in Pulmonary Ventilation Associated with an Auditory Stimulus (a bell)

Day of observation				
1st	2nd	3rd	4th	5th
		(Pulmonary ventilation, in liters)		
1.0	0.2	0.4	0.7	1.6

As appears from Table 1, auditory stimulation (a bell) at first augments pulmonary ventilation, but this is followed by its diminution to values below those found initially, before stimulation was instituted.

Passive movements of the left arm also caused augmentation of pulmonary ventilation and of oxygen uptake, but this increase varied within certain limits, without manifesting a tendency towards diminution (Table 2).

It thus appears that the actions of the above-mentioned stimuli, applied both to rested and to fatigued individuals, manifest definite differences with respect to their effect on working capacity and on the effecting of reactions which enable muscular work to be done.

TABLE 2

Changes in Pulmonary Ventilation and Oxygen Uptake, With Passive Movements of the Left Arm

Index measured	Day of observation						
	1st	2nd	3rd	4th	5th	6th	7th
Pulmonary ventilation (in liters)	0.7	0.8	0.3	0.5	0.6	1.6	1.3
Oxygen uptake (in ml)	33	34	57	48	56	43	30

Each of the stimuli was a novel factor in the given situation when first applied, and could, according to the concepts of I. P. Pavlov, evoke an orientating reaction: "What is it?" The stimulator of the auditory analysor applied by us initially evoked a generalized state of alertness, as probably would any other fresh stimulus. This brought about an increase in pulmonary ventilation and in the amount of work performed. When the stimulation experiments were repeated, the effects were evidently concentrated in the analysor to which they were directed. The subject continued to hear the bell, but this no longer caused any change in work capacity, nor did it augment pulmonary ventilation.

Pulmonary ventilation rose the first few times the bell was sounded, but after a few repetitions the effect was inverted. In other words, the factor which initially had a stimulating effect became, after a few repetitions, an inhibitory one. This effect may be due to inductive inhibition, arising as a consequence of concentration of excitation. As has been shown by N. A. Popov [8], this type of reaction is characteristic of the orientating reflex.

With stimulation of the motor analysor, effected by movements of the arm, the stimulus passes directly to this analysor, increased excitation of which leads to increased work performance. It follows that, even after concentration of the initially generalized state of excitation, supplementary stimulation of the same motor analysor will take place, and this can apparently be maintained by irradiation from other centers in which excitation may arise as a result of stimuli from proprioceptors. The existence of excitation of this sort has been confirmed by the changes in pulmonary ventilation and gaseous metabolism observed when proprioceptors are stimulated. Working with cats, D. G. Kvasov and I. I. Naumenkov [4] have shown that direct stimulation of motor nerves supplying muscles leads to increase in pulmonary ventilation and blood pressure, even in the absence of muscular contraction.

The ability of proprioceptors to respond for a long time to stimulation by impulses of constant strength may be an additional factor favoring stabilization of the given reaction.

SUMMARY

The stability of changes of the muscular working capacity under the effect of various analysors was investigated in this work. This study was conducted on the background of decreased working ability of the right arm.

The results of these observations showed that increase of working capacity caused by stimulation of the cutaneous temperature and olfactory analysors disappears following repetition of these experiments 2 or 3 times. They are components of reaction of orientation. Increase of the working capacity under the effect of additional stimulation of the motor analysor is very stable under conditions of these experiments. Evidently, excitation of certain centers plays an important part in creation of stability of this effect. This excitation appears as an unconditioned reflex as a result of stimulation of the proprioceptors.

LITERATURE CITED

[1] V. O. Bogoslavskii, Curve representing Muscle Fatigue in the Human under Various Conditions,* (Thesis), St. Petersburg, 1891.

[2] N. K. Vereshchagin and V. V. Rozenblat, Biull. Eksptl. Biol. i Med. 34, No. 9, 19-22 (1952).

[3] G. V. Gladoschuk in: Study of Regulation of Physiological Functions under the Natural Environmental Conditions of the Organism, * Vol. 3, 323-333, Moscow-Leningrad 1954.

[4] D. G. Kvasov and I. I. Naumenkov, Biull. Eksptl. Biol. i Med. 41, No. 1, 27-31 (1951).

[5] N. I. Konopasevich, Further Contributions to the Physiology of Muscle Fatigue in the Human (Thesis),* St. Petersburg 1892.

[6] V. B. Liberman and G. A. Trubitsyna, "Study of Regulation of Physiological Functions under the Natural Environmental Conditions of the Organism,"* Vol. 3, 259-273, Moscow-Leningrad 1954.

[7] E. E. Plotnikova and A. A. Kniazeva in: Advances in Reflexology and in the Physiology of the Nervous System,* Vol. 3, 129-137, Moscow-Leningrad 1929.

[8] N. A. Popov, Russ. Fiziol. Zhur. 3, Nos. 1-5, 10-13 (1921).

[9] I. M. Sechenov, Collected Works, Vol. 1,* 244-261, Moscow 1907.

[10] K. M. Smirnov, Study of Regulation of Physiological Functions under the Natural Environmental Conditions of the Organism,* Vol. 3, 274-310, Moscow-Leningrad 1954.

[11] D. I. Shatenshtein, and E. N. Iordanskaia, Fiziol. Zhur. 41, No. 1, 35-41 (1955).

* In Russian.

REFLEX EFFECT ON THE STOMACH OF VIBRATIONS PROCEEDING FROM THE HEART*

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The existence of cardiac reception has now been finally confirmed. Animal experiments have shown the existence of reflexes from the myo- and pericardium, causing retardation or arrest of heart-beat, respiratory changes [3, 7, 11], and also initiation of reflexes affecting skeletal muscles [6, 12]. The present paper deals with reflex action of the heart on the stomach, evoked by mechanical (vibratory) stimulation of the heart.

In our earlier studies of the effects of vibratory stimulation of the heart and stomach [8-10] we encountered the following phenomenon: application of the vibrator to the heart of a frog not only affected the activity of the heart, but also affected the stomach, the motor activity of which was modified. In order to study this effect in greater detail, we performed a series of special experiments on frogs, involving application of a vibrator to the heart, with simultaneous observation of the motor activities of the stomach.

EXPERIMENTAL METHODS AND RESULTS

Our experiments were performed on Rana temporaria. After transection above the level of the medulla oblongata we destroyed the brain. Kymograph recordings were made of contractions of the heart and stomach in situ. Stimulation was effected by means of an electromagnetic vibrator, of amplitude 0.3 mm and frequency 100 vibration per second; the rod of the vibrator was applied closely to the heart. The duration of action of the stimulator was from 1 to 3 minutes.

The reflex effects on the stomach observed in 67 experiments in which the vibrator was applied to the heart are shown in the Table.

Changes in Gastric Motor Activity due to the Action of a Vibrator on the Heart

Nature of changes in gastric motor activities	Number of experiments	Percentage of total number of experiments
Increased frequency of peristaltic waves and increase in their amplitude	15	23
Increased frequency of peristaltic waves without change in amplitude	25	38
No change in frequency, but increase in amplitude	5	7
Appearance of marked peristalsis, which was absent before application of the vibrator	9	13
No change in peristalsis, but heightening of gastric tonus	11	16
Retarded peristalsis	2	3

* Read at the IX Scientific Conference of the Perm Medical Institute, March 10, 1949.

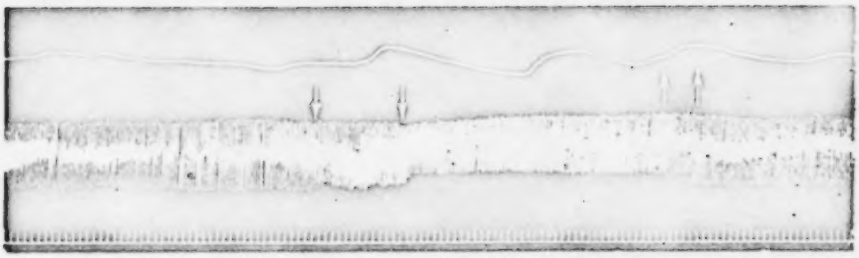

Fig. 1. Heightened gastric peristalsis due to the action of vibration on the heart. Explanation of tracings (from above down): recording of gastric contractions, recording of heart contractions, time marker (5 seconds); ↓ contact of the vibrator with the heart; ↓ beginning of vibration; ↑ cessation of vibration.

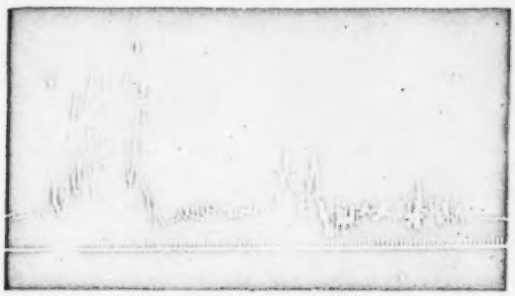

Fig. 2. Contractions of the fasting stomach of a dog subjected to the action of a vibrator placed over the heart. The arrows indicate when the vibrator was switched on and off; time marker below (5 seconds).

As is seen from the table, in 82% of all the experiments, stimulation of the heart by the vibrator caused heightened peristalsis of the stomach (Fig. 1), manifested as more frequent or more energetic contractions of the stomach muscles. The latent period of the gastric reaction amounted to 5-10 seconds after application of the vibrator to the heart, but in some experiments it amounted to more than 30 seconds.

In 65% of the experiments there was a rise in gastric tonus, and no perceptible change in 35% of the experiments. The heightening of gastric tonus persisted for 15-20 minutes or more after removal of the vibrator.

We stimulated the heart in 9 experiments in which gastric peristalsis was totally absent (as shown by the kymograms). In all these cases, a lively peristalsis appeared after application of the vibrator, and continued to increase in intensity after its removal. These experiments afforded particularly clear evidence of the reflex action of the heart on the stomach.

In certain of the experiments we observed displacement of the entire stomach after applying the vibrator. This can only be due to a reflex from the heart acting on skeletal muscles (a visceromotor reflex).

Since the heart was located in the vicinity of the stomach during the in situ experiments, the possibility existed that the vibrations might reach the stomach via the intervening tissues. If this were to be the case, we would not be justified in concluding that the changes in gastric activity observed after applying the vibrator to the heart were of reflex origin. In order to elucidate this question, we performed an additional 40 experiments on the effect on the stomach of vibration applied to the heart, on animals in which we had destroyed different parts of the central nervous system, beginning with the cerebral cortex, and ending up with the spinal cord, observing the effect on the motor activity of the stomach. The results of these experiments were as follows:

1) when the brain was destroyed above the level of the medulla oblongata, and also when the spinal cord was additionally destroyed below the level of the first cervical vertebra, leaving the medulla oblongata intact, the effect of vibration applied to the heart was, in 78% of the experiments, to stimulate persistalsis of the stomach, which was unaffected in the remaining 22%;

2) with destruction of both the brain and the medulla, i.e., spinal frogs, in no case did application of vibration to the heart affect gastric peristalsis;

3) after destruction of the entire central nervous system (including the spinal cord) application of the vibrator to the heart also had no effect on gastric motor activity.

It thus appears that the heightening of gastric peristalsis observed when a vibratory stimulus is applied to the heart takes place only when the medulla oblongata is intact; after its destruction, stimulation of the heart does not affect gastric motor activity. It may hence be concluded that the stimulation of gastric motor activity observed in our experiments cannot be ascribed to mechanical transmission of vibration from the heart to the stomach through the surrounding tissues, but must be of reflex nature. This reflex must be effected via the medulla oblongata.

Our experiments on poikilotherms were next extended to dogs provided with a chronic gastric fistula. The experiments began with the recording on a kymograph, using V. N. Boldyrev's method [2], of periodic hunger contractions of the stomach, giving records of the duration and the characteristics of the periods of activity and rest. The vibrator was then applied to the cardiac region of the dog, for periods of 2 to 10 minutes. The vibrator (frequency 100 cps, amplitude of vibrations 0.3 mm) was fastened to the left side of the chest, over the heart, before the experiment was begun. The vibrator was switched on at different phases of the periodic activity of the stomach, viz., at rest, and at the beginning and end of periods of activity.

The results of 30 experiments showed that the stomach reaction varied according to its initial functional state. We were never able to evoke contractions when the stomach was in a fully quiescent phase. Nor did stimulation of the heart applied during an active phase have any significant effect on the motor activity of the stomach. However, stimulation of the heart 4-8 minutes after cessation of a periodic contraction phase caused renewal of contractions in 9 out of 10 experiments (Fig. 2).

In order to establish that this reaction of the stomach was of reflex origin, and that the reflex proceeded from the heart, and not from the skin, we performed additional experiments on the same dog, in which we placed the vibrator on the right side of the chest, at a point corresponding with respect to its spinal innervation to the previous location over the heart. These experiments did not give rise to any well-defined reflex reactions of the stomach. We may hence conclude that the stomach reaction observed by us when the vibrator was placed over the heart was caused by a reflex from the heart, and not from the skin.

The experiments on dogs thus confirm the results obtained with poikilotherms; the reflex from the heart may also be demonstrated in homeotherms, which is of definite interest for the study of the physiology of interoception. This reflex reaction should be more pronounced in pathological conditions of the heart, and this has been confirmed by clinical observation [1, 4, 5], showing that diseases or lesions of the heart cause dyspepsia, and sometimes present the clinical picture of gastric ulcer, cholecystitis, acute abdomen, etc.

SUMMARY

The effect of heart stimulation by vibrations (with the aid of electromagnetic vibrator) on the motor function of the stomach was studied in experiments on animals (frogs and dogs).

LITERATURE CITED

[1] N. K. Bogolepov, Sovetskii Vrachebnyi Sbornik No. 13, 7-9 (1949).

[2] V. N. Boldyrev, "Periodic Functioning of the Digestive Apparatus with a Fasting Stomach" (Thesis),* St. Petersburg 1904.

[3] P. P. Goncharov, Tamponade of the Heart * Leningrad, 1936.

[4] P. T. Kazakov and I. P. Fridliand, Terap. Arkh. 15, No. 4, 596-603 (1937).

* In Russian.

[5] G. P. Kovtunovich, Jubilee Collection of Scientific Papers of the Leningrad Refresher Course Inst. for Med. Practitioners, pp. 299-314,* Leningrad 1935.

[6] M. R. Mogendovich, Sensitivity of Viscera, and the Chronaxie of Skeletal Musculature* Leningrad, 1941.

[7] V. I. Popov, Trudy Sverdlovsk. Med. Inst. 15, 195-279 (Sverdlovsk 1941).

[8] V. P. Riumin, Biull. Eksptl. Biol. i Med. 26, No. 1, 126-130 (1948).

[9] V. P. Riumin, Biological and Therapeutic Action of Magnetic Fields and of Strictly Periodic Vibrations pp. 103-114, Perm. 1948.*

[10] V. P. Riumin, Collection of Sci. Works of the Perm. Med. Inst. pp. 32-40, Perm. 1955.

[11] V. N. Chernigovskii, Biull. Eksptl. Biol. i Med. 15, No. 1-2, 43-45 (1943).

[12] G. Z. Chuvaeva, Collection of Sci. Works of the Perm. Med. Inst., pp. 23-31, Perm. 1955.

* In Russian.

PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

INVESTIGATION OF THE MECHANISM UNDERLYING THE PHYSIOLOGIC ACTION OF ARTIFICIAL CLOSED PNEUMOTHORAX

COMMUNICATION II. PULMONARY BIOMECHANICS IN ARTIFICIAL CLOSED PNEUMOTHORAX

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It was shown in the previous communication that artificial closed pneumothorax elicited substantial changes in efferent impulses in the pulmonary branches of the vagus and consequently in the activity of the respiratory center. These data, together with facts described in the literature [1, 4, 6, 9], suggested that an important role in the mechanism of the influence exerted by artificial pneumothorax was played by reflex factors.

This question has not received adequate experimental examination and requires investigation of the concrete mechanisms underlying the functional changes in external respiration during pneumothorax.

The present communication is concerned with the study of pulmonary biomechanics of pneumothorax (by means of investigation and comparison of the distinctive features of respiratory movements of the thorax, intrapleural and intratrachael pressure, magnitude of pulmonary ventilation and arterial blood pressure) at the moment of induction of closed experimental pneumothorax and during adjustment to it.

EXPERIMENTAL METHOD

Two series of experiments were carried out: short-term experiments on 16 cats and prolonged experiments on 2 dogs.

In the experiments on urethane-anesthetized cats (1 g/kg urethane) a study was made of the effect on induced closed pneumothorax and of subsequent aspiration of air from the pleural cavity, on the dynamics of respiratory movements of the thorax, changes in its volume, air pressure in the trachea, intrapleural pressure and arterial blood pressure.

Thoracic respiratory movements were recorded by means of a crimped rubber tube fastened around the thorax and connected with a Marey's tambour. This method of recording respiratory movements permits observation of changes in the mid-position of the thorax and hence relative changes in its volume can be judged.

Fluctuations of air pressure in the trachea during the respiratory phases were recorded by the usual method by means of a tracheal cannula connected to a Marey's tambour. Intrapleural pressure was recorded by means of a system consisting of a hollow needle which was introduced into the pleural cavity, rubber tubes and a water manometer connected to a Marey's tambour. Blood pressure was recorded in the common carotid artery by a mercury manometer and a tonometer. In some experiments the other carotid artery was also exposed. In the course of the experiment a clamp was placed on it for 10-15 seconds from time to time in order to determine the level of reflex excitability of the vasomotor center.

Introduction of air into the pleural cavity and aspiration of air from the latter were achieved by means of a pneumothorax apparatus which was connected by a T-joint to the intrapleural pressure recording system.

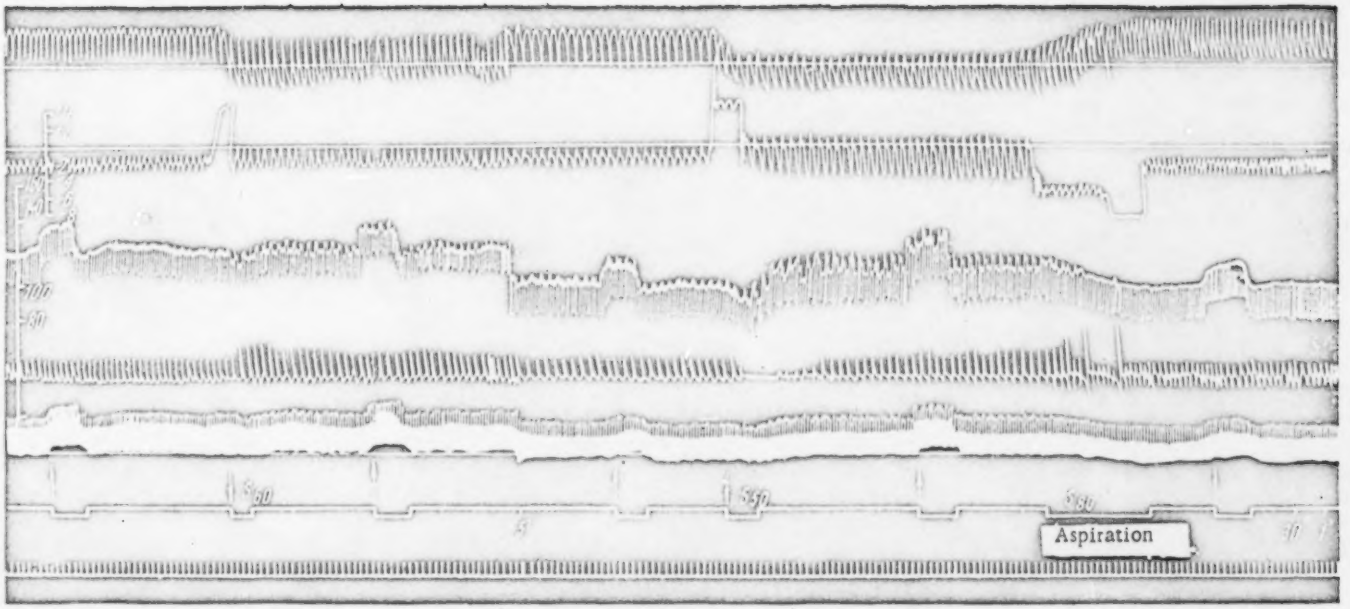

Fig. 1. Dynamics of the changes in thoracic volume, intrapleural pressure, respiration and blood pressure during induction of closed pneumothorax and during aspiration of air from the pleural cavity.

Cat weighing 3.1 kg. Data of experiment September 30, 1954. Records (from above down): respiratory movements of the thorax (inspiration – downward deflection), intrapleural pressure and its base line, blood pressure (mercury manometer), respiration (tracheal record), blood pressure (tonometer), mercury manometer base line, signal line (↓ S_{60} – 60 ml air introduced into the left pleural cavity; ↓ S_{50} – ditto, 50 ml; asp. S_{80} – aspiration of 80 ml air from the pleural cavity; ↑ – compression of the left carotid artery; figures underneath the signal line – time period during which the kymograph was stopped; time marker (2 seconds).

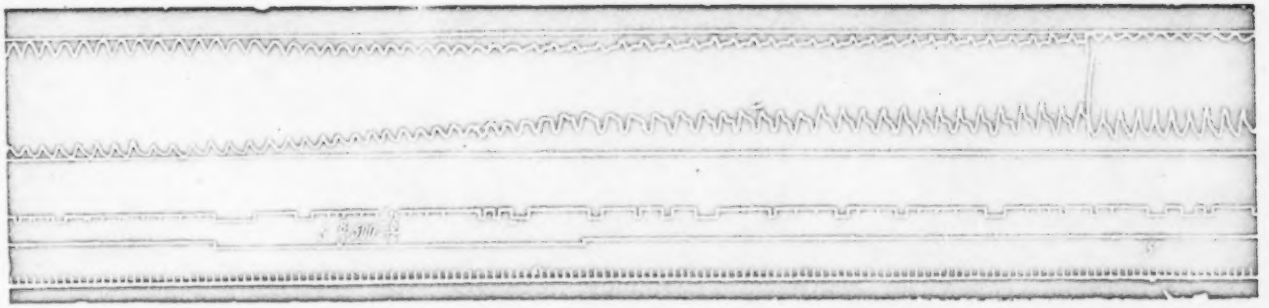

Fig. 2. Compensatory expansion of the thorax, change in the form of the respiratory curve and in the magnitude of pulmonary ventilation upon induction of closed pneumothorax.
Experiment on the dog Prim, March 11, 1955. Records (from above down): pneumogram (abdominal respiration – inspiration indicated by downward deflection), pneumogram (thoracic respiration – inspiration denoted by upward deflection); pulmonary ventilation (one mark corresponds to 200 ml inspired air); mark denoting induction of pneumothorax; time marker (1 second).

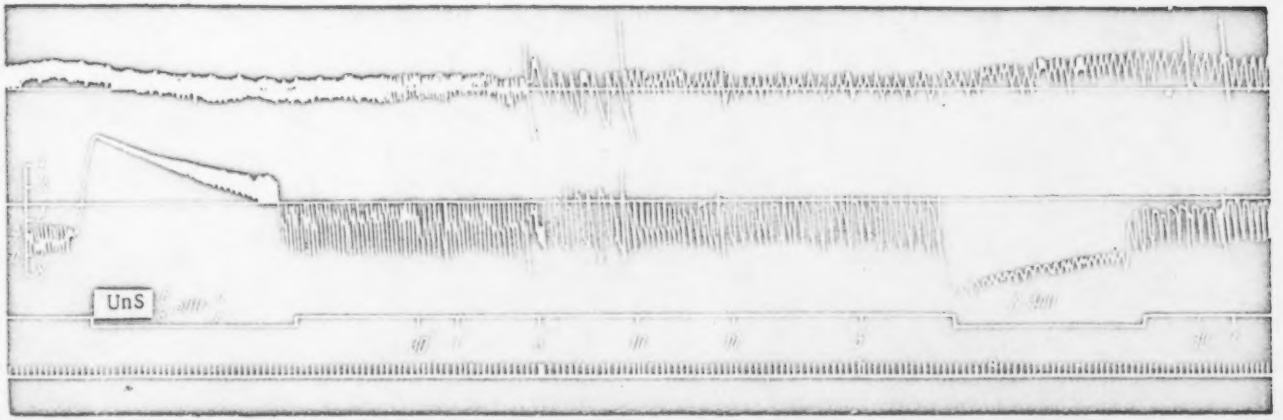

Fig. 3. Changes in thoracic volume and intrapleural pressure upon induction of closed pneumothorax and on subsequent aspiration of air from the pleural cavity. Dog Chernushka, weighing 23 kg. Date of experiment October 7, 1954.
Records (from above down): pneumogram (inspiration – downward deflection, intrapleural pressure and its base line, signal line (UnS_{500} – 500 ml air introduced into the left pleural cavity; asp. S_{400} – 400 ml air aspirated from the left pleural cavity; figures underneath the signal line – period of time during which the kymograph was stopped; time marker (2 seconds).

In the second series of experiments, performed on the dogs Prim and Chernushka, kymographic records were made of the respiratory movements of the thorax and the abdomen, intrapleural pressure and the magnitude of pulmonary ventilation. A hermetic mask was placed on the dog's muzzle in order to record pulmonary ventilation. The expired air passed from the mask by a connecting tube to a gas meter, the movement of whose needle was recorded on the kymograph by means of electric contacts and an electric marker. The marker recorded a mark for every 200 ml of air which passed through the gas meter.

EXPERIMENTAL RESULTS

When 30-60 ml of air was introduced into the intrapleural space of cats (weighing 2.5-3.5 kg) substantial changes were recorded in the interrelation and functions of the intrathoracic organs. The kymograms show (Fig. 1) that immediately after induction of pneumothorax the increase in intrapleural pressure is accompanied by considerable increase of inspiratory tonus of the thoracic respiratory musculature. The thorax passes into the position of inspiration as it were, but its respiratory excursions continue and their amplitude not only does not diminish but is even enhanced to some extent. The volume of the thorax thus increases following induction of pneumothorax.

The amplitude of the curve recorded from the trachea under these experimental conditions shows an increase. The blood pressure in the carotid artery drops by 5-10 mm Hg at the moment of induction of pneumothorax and then returns to the initial level or rises by 10-15 min Hg.

After a certain time has elapsed from the induction of pneumothorax the respiration and circulation settle down at a level somewhat different from the initial one. The inspiratory tonus of the thorax gradually decreases while intrapleural pressure, despite the presence of air in the pleural cavity, displays a tendency to return to the initial level characteristic for the given animal.

Following additional introduction of air into the pleural cavity and creation of a more "tense" pneumothorax (Fig. 1, S_{50}) the inspiratory tonus of the thorax becomes still more marked. The amplitude of the respiratory curve recorded from the trachea at the moment of induction of pneumothorax drops to zero and then increases gradually beyond the initial level. This phenomenon is apparently determined by changes in the lumen of the air passages.

The blood pressure in "tense" pneumothorax shows a small fall at the moment of pneumothorax induction followed by a rise when fluctuations associated with respiratory phases become considerably enhanced.

Following aspiration of air from the pleural cavity, respiration and blood pressure gradually return to normal (Fig. 1, S_{80}).

The phenomenon of increased inspiratory tonus of the thorax and the increase in its mean volume after induction of pneumothorax described above has also been observed after preliminary section of the phrenic nerves in the neck. The effect was diminished but not abolished by bilateral vagotomy.

In the long-term experiments on dogs, data similar to those given by the short-term experiments on cats were obtained. The kymograms show (Figs. 2 and 3) that induction of closed pneumothorax is accompanied by a compensatory expansion of the thorax and changes in the inter-relations of the respiratory phases. Not infrequently respiratory arrest at the beginning of expiration was noted at the moment of induction of pneumothorax (Fig. 2).

There is some decrease in pulmonary ventilation and depth of inspiration at the moment of pneumothorax induction and immediately following it, but after only a few minutes the pulmonary ventilation returns to normal or even exceeds that value. The latter indicates that the lungs execute considerable respiratory movement after induction of pneumothorax. The intrapleural pressure in the experimental dogs returned to normal 2-3 days after the induction of pneumothorax, i.e., before the air in the pleural cavity was absorbed.

DISCUSSION

The results obtained show that artificial closed pneumothorax elicits substantial changes in respiration, and, within certain limits, promotes the appearance of a number of compensatory-adaptive reactions of the organism as a whole. There is no doubt that in "unstrained" pneumothorax collapse of the lung is not the main and significant factor in the mechanism of pneumothorax action.

The fact noted by us, viz. that the intrapleural pressure returns to the initial level before the absorption of the air from the pleural cavity, agrees with the data obtained by a number of authors [1, 2, 4, 5, 10, 11] who also observed inconsistencies between the volume of air introduced into the pleural cavity and the magnitude of change in intrapleural pressure.

F. Parodi [11] had used this fact to prove his "theory of pulmonary weight." Other authors regarded it as the basic argument for postulating a theory of pleuro-pulmonary reflex [1, 4, 5, 9].

We conclude on the basis of our experiments that the inconsistency between the volume of air introduced and intrapleural pressure during pneumothorax is the result of compensatory expansion of the thorax elicited by a reflex from the pleural and pulmonary interoceptors affecting the tonus of respiratory musculature. Under conditions of pneumothorax this reflex ensures adaptive changes in the inter-relation of the mean volume of the thorax and volume of the lungs. The possibility of reflex changes in the tonus of bronchial muscles and pulmonary blood vessels must also be kept in mind [3, 7, 8, 10, 12, 13].

The biologic significance of these reflexes should be considered in terms of the importance of maintaining a relative physiologic equilibrium in the system's respiratory apparatus – intrapleural pressure. This equilibrium must be referred to a number of such finely regulated body constants as the physicochemical composition of the blood, body temperature, blood pressure etc.

The tendency of the lung to expand to its natural volume after induction of pneumothorax, as well as the fact of absorption of air from the pleural cavity, favor this concept.

It is not surprising that these reflexes are masked under physiologic conditions and are not immediately detectable. When the organism is placed under extreme conditions these reflexes tend to appear more clearly.

SUMMARY

Short-term experiments were performed on cats and prolonged ones on dogs. It was established that in "unstrained" experimentally-induced closed pneumothorax intensive respiratory movements were performed by the lungs. Pneumothorax causes compensatory expansion of the thorax by reflex mechanism which acts from interoceptors of the pleura and lungs on the tone of the respiratory muscles.

Reflex regulation of the average volume of the thorax and the ratio of the volume of the thorax to that of the lungs is postulated.

LITERATURE CITED

[1] M. Ia. Babitskii, In the book: Investigation of the Role of the Nervous System in the Pathogenesis and Treatment of Tuberculosis.* Moscow, 1954, pp. 295-293, 302-308.

[2] M. V. Ikonen and Ia. G. Uzhanskii, Problemy Tuberk., No. 1, 28-35 (1941).

[3] M. E. Marshak, Biull. Eksptl. Biol. i Med., No. 2, 4-8 (1953).

[4] F. A. Mikhailov, Klin. Med., No. 6, 21-26 (1951).

[5] L. R. Perel'man, Transactions of the 5th All-Union Congress of Medical Tuberculosis Specialists,* Moscow, 1950, pp. 92-100.

[6] V. A. Ravich-Sherbo, Tuberculosis in Adults,* Moscow, 1953.

[7] V. A. Ravich-Sherbo and E. E. Klebanova, Problemy Tuberk., No. 2, 11-19 (1952).

[8] T. M. Turpaev and T. G. Putintseva, Fiziol. Zhur. SSSR, 41, No. 1, 71-77 (1955).

[9] Ia. G. Uzhanskii, Arkh. Patol., No. 4, 3-8 (1947).

[10] K. Bucher and U. Lanz, Schweiz. Ztschr. f. Tuberk., 1954, Vol. 11, No. 2, pp. 146-152.

* In Russian.

[11] F. Parodi, Hypotension-Induced Pulmonary Rest in the Treatment of Pulmonary Tuberculosis, Tashkent, 1938).

[12] E. Reinhardt, Arch. path. Anat. 1934, Vol. 292, pp. 322-355.

[13] F. Verzar and L. Jaker, Arch. f. ges. Physiol. 1936, Vol. 238, pp. 379-386.

VITAMIN B_{12} CONTENT OF THE BLOOD OF DOGS DEVELOPING ANEMIAS AFTER THE FORMATION OF SMALL STOMACH POUCHES USING THE METHOD OF KLEMENTSIEVICH-HEIDENHAIN

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Many investigators have shown that denervation of some internal organs in animals will produce various types of anemia [2, 3, 4, 7, 8, 9, 11, 12].

This phenomenon has been viewed as a consequence of the disappearance of afferent and efferent impulses.

R. A. Durinian [2], O. M. Preger [7 and 8], S. I. Iakovlev [12] and others have shown that partial gastric denervation produced by the formation of a small stomach pouch employing the method of Klementsievich-Heidenhain will cause in animals the development of a macrocytic hyperchromic anemia of the hyporegenerative type.

The studies of N. A. Fedorov and his co-workers [10], S. I. Iakovlev [12] have also demonstrated that the gastric juice obtained from these Klementsievich-Heidenhain pouches in dogs in the period of hyperchromic anemia had very little of the anti-anemic factor, it being at times completely absent.

It is universally agreed that vitamin B_{12} is a very potent "external" anti-anemic factor. The utilization of vitamin B_{12} by the organism is closely associated with normal secretory activities of the mucous membranes of the entire gastrointestinal tract and to a large degree of the stomach itself.

We sought an answer to the question of whether the anemia so produced was caused by a disturbed absorption into the blood stream of the vitamin B_{12}; we therefore conducted experiments during the course of which we determined the level in the blood serum of the vitamin in dogs which had partially denervated stomachs.

EXPERIMENTAL METHODS

Under the conditions of a chronic experiment the blood picture was studied in male dogs, before and after the operation. As indicators of the conditions of the blood in the animals, counts were made of hemoglobin and vitamin B_{12} and the numbers of erythrocytes and leucocytes.

The blood was drawn from the fasting animal's femoral vein into a sterile centrifuge tube for vitamin B_{12} determinations and into ordinary test tubes for later use in Sahli hemoglobinometer and w.b.c. and r.b.c. determinations.

When calculating the color index for each animal we used the averages obtained for the hemoglobin and erythrocytes before the operation. This was done in an attempt to duplicate the methods and conditions of the work of R. A. Durinian [2]; using his findings as a guide, we found it necessary to perform completely only the biochemical studies.

The blood drawn from the vein under sterile conditions was centrifuged; to the serum was added an equal volume of acetate buffer, pH 4.6, and 4 volumes of distilled water [6]. After boiling for 1/2 hour on a water bath,

the protein precipitates were separated by centrifuging and the vitamin B_{12} content of the clear supernatant liquid was determined. The microbiological method of determining vitamin B_{12} was employed [1]. As the test microbe we used the intestinal bacillus whose original strain we obtained from the laboratory of Prof. V. I. Bukin. This strain of the intestinal bacillus, when seeded on a liquid medium containing vitamin B_{12}, will grow and cloud the solution in direct proportion to the amount of contained vitamin B_{12}. The degree of turbidity was determined in a photoelectrometer FEK-M, using a blue light filter.

Three dogs were studied for four weeks in order to observe the normal range of variations, after which they were operated and Klementsievich-Heidenhain gastric pouches were made. One dog served as a control and was not operated. It should be stated that the pouches were made much larger than usual so that almost the entire body and all of the bottom of the stomach was included. The postoperative studies were conducted for 5 months. The blood studies were begun 40 days after the operation by which time the postoperative anemia had disappeared and the specific hyperchromic macrocytic anemia had begun to develop.

EXPERIMENTAL RESULTS

Our studies demonstrated that 40-60 days after the formation of the Klementsievich-Heidenhain pouches the blood of the dogs underwent observable changes while in the control animal the variations stayed within normal limits, i.e., 2-4% Hb and up to 1/2 million erythrocytes.

In the three operated dogs the amount of hemoglobin dropped an average of 11.8%, the numer of erythrocytes diminished by an average 1,180,766, while the number of leucocytes increased by 2968.

It could be supposed that along with the lowering of the hemoglobin and decrease in erythrocytes the blood of the dogs would show also a decrease in the amount of vitamin B_{12}. However, in two of the operated dogs, Bars and Dobry, the amount of B_{12} did not diminish, but remained on the preoperative level. As an example, the results with dog Bars as presented in Table 2 may be studied. Table 2 demonstrates that the developing anemia is not paralleled by a decrease of the blood level of B_{12} so that, apparently, it does not depend on any disturbance in the absorption of vitamin B_{12} from the outside.

In the third dog, Ryzhik, Table 3 shows a 3.2-fold decrease in the blood level of vitamin B_{12} at a time when the number of erythrocytes and amount of hemoglobin diminished within approximately the same limits as shown for the first two dogs. It should be noted that the dog Ryzhik had a severe irritation of the skin of the abdomen associated with rather luxuriant granulation tissues all due to the constant copious flow of gastric juice from the gastric pouch. For this reason we could not assume that the above described phenomena were due solely to the presence of a gastric pouch. Still, we considered that the anemia and decrease of vitamin B_{12} in Ryzhik was produced by disturbance in B_{12} absorption. In that case the blood picture could be altered by intramuscular injections of vitamin B_{12}.

Vitamin B_{12} in amount of 15 γ was given intramuscularly to all the dogs every third day for 4 weeks.

The results of these experiments showed that in all the dogs except Ryzhik, injection of vitamin B_{12} after the first or second injection increased the amount of hemoglobin and the blood erythrocyte count which then stabilized at the higher levels.

However, in the operated dogs Bars and Dobry the amount of hemoglobin and the number of erythrocytes did not reach preoperative levels. In the control dog the injection of vitamin B_{12} caused a rise in the figures for hemoglobin and erythrocytes which lasted 4-6 days after which the indicators returned to the base levels existing before the introduction of vitamin B_{12}.

Along with the rise in the amount of hemoglobin and number of erythrocytes, the number of leucocytes diminished after the introduction of the vitamin B_{12}, falling even below the preoperative level.

The operated animals failed to show a rise in the blood level of the vitamin B_{12} after the first two injections while the later injections gave a noticeable increase. When the tables are examined carefully, it can be observed that the vitamin B_{12} level in the blood of the control animal (Table 1) differed rather markedly from the corresponding figures for the operated animal (Table 2) throughout the period of injecting the vitamin. In the control dog, Sever (Table 1) during this period, the average for the serum content of B_{12} did not differ from the figures before the treatment (0.359-0.329 m μg), while at the same time in the dogs having the gastric pouch a marked

TABLE 1

Alterations in the Composition of the Blood and the Vitamin B_{12} Content in the Control Dog Sever

Date of study	hemo-globin	erythro-cytes	color index	leuco-cytes	Vitamin B_{12} content in m μg/cc
19/VI 1956	78%	6 500 000	1.0	10 000	0.356
2/VI 1956	76%	6 800 000	0.96	9 000	0.402
22/VI 1956	77%	6 400 000	0.98	9 500	0.379
3/VII 1956	76%	—	—	8 750	0.400
Average	76.7	6 570 000		9 312	0.384
29/VIII 1956	80%	6 900 000	0.98	8 750	0.374
5/IX 1956	80%	6 800 000	0.99	9 500	0.374
13/IX 1956	80%	6 800 000	0.99	10 000	0.396
17/IX 1956	82%	6 900 000	0.99	—	0.396
27/IX 1956	78%	6 700 000	0.98	9 625	0.339
2/X 1956	78%	6 700 000	0.98	—	0.300
10/X 1956	72%	6 400 000	0.94	10 000	0.149
17/X 1956	80%	6 820 000	0.97	9 750	0.305
Average	78%	6 752 000	—	9 604	0.329
23/X 1956					[1]
26/X 1956	85%	7 540 000	0.96	8 100	0.249[1]
30/X 1956	85%	7 500 000	0.96		—[1]
2/XI 1956					[1]
5/XI 1956	80%	—	—	—	0.375[1]
9/XI 1956	80%	6 900 000	0.98	10 000	0.373[1]
12/XI 1956					[1]
13/XI 1956	80%	6 800 000	0.99	8 170	0.438[1]
16/XI 1956					[1]
19/XI 1956	80%	6 840 000	0.99		0.413[1]
22/XI 1956	80%	7 040 000	0.96	8 750	0.309
Average	81%	7 103 000		8 750	0.359
27/XI 1956	78%	6 800 000	0.98		0.309

[1] Intramuscular injection of the 15 γ of vitamin B_{12}.

difference was seen. Thus, for example, in dog Dobry the difference was +0.122 m μg while in Bars it was +0.173 m μg.

Table 1 shows that in the control animal the vitamin B_{12} level approached base level 2-3 days after the injection while in the operated animals 4-5 days elapsed after the injection before normal levels were reached. This lag is due, apparently, to a disturbance in the process of depot storage and utilization of vitamin B_{12} in the operated animals.

This idea has been expressed previously by I.B. Likhtsier and his co-workers [5] who investigated the amount of vitamin B_{12} present in the blood and urine of patients having cirrhosis of the liver and chronic enterocolitis with complicating anemia. Within an hour after intramuscular introduction of vitamin B_{12} these

TABLE 2

Alterations in the Composition of the Blood and the Vitamin B_{12} Content in Dog Bars with a Partially Denervated Stomach

Date of study	hemoglobin	erythrocytes	color index	leucocytes	Vitamin B_{12} content in m μg/cc
12/VI 1956	93%	7 500 000	0.95	9 000	0.400
19/VI 1956	89%	7 000 000	1.0	10 000	0.310
26/VI 1956	89%	7 080 000	0.99	8 750	0.381
3/VII 1956	93%	7 220 000	1.02	9 000	0.400
Average	91%	7 200 000		9 187	0,372
10/VII 1956 Date of operation when the Klementsievich – Heidenhain pouch was made					
22/VIII 1956	69%	5 160 000	1.07	17 500	—
29/VIII 1956	73%	5 640 000	1.03	15 000	0.320
5/IX 1956	76%	6 000 000	1.00	15 125	0.320
13/IX 1956	78%	5 900 000	1.04	9 000	0.418
17/IX 1956	80%	6 300 000	1.00		0.534
27/IX 1956	73%	5 600 000	1.03	10 500	0.378
2/X 1956	74%	5 800 000	1.00	9 000	0.377
10/X 1956	75%	5 620 000	1.05	—	0.387
17/X 1956	73%	5 656 000	1.03	10 500	0.301
Average . .	74.5%	5 741 700		12 364	0,379
23/X 1956					[1]
26/X 1956	85%	6 500 000	1.03	—	0.356[1]
30/X 1956	85%	6 600 000	1.03	7 000	0.350[1]
2/XI 1956					[1]
5/XI 1956	82%	6 340 000	1.03	6 800	0.520[1]
9/XI 1956	82%	6 340 000	1.03	6 700	0.513[1]
12/XI 1956					[1]
13/XI 1956	80%	6 040 000	1.05	6 900	0.600[1]
16/XI 1956					[1]
19/XI 1956	80%	6 000 000	1.05		0.520[1]
22/XI 1956	80%	6 000 000	1.05	9 750	0.505[1]
Average . .	82%	6 260 000		7 430	0.552
27/XI 1956	80%	6 000 000	1.05		0.480
7/XII 1956	80%	6 000 000	1.05		0.390

[1] Intramuscular injection of 15 γ of vitamin B_{12}.

authors observed in these patients a sharp rise in the content of the vitamin in the blood. The B_{12} level in the serum of these patients did not return to base level even after 3 days.

In our experiments with dog Ryzhik, in spite of the introduction of vitamin B_{12} and the rise of its blood level, the r.b.c. count and the amount of hemoglobin continued to fall. In this specific instance it might be thought that the secondary manifestations such as the large inflammatory surface of the skin of the animal had more influence on the hemogram in general and the vitamin B_{12} level in particular than the presence of a gastric pouch.

TABLE 3

Alterations in the Composition of the Blood and the Vitamin B_{12} Content in Dog Ryzhik with a Partially Denervated Stomach

Date of study	hemo-globin	erythro-cytes	color index	leuco-cytes	Vitamin B_{12} content in m μg/cc
12/VI 1956	74%	6 000 000	0.97	11 750	0.244
19/VI 1956	76%	6 200 000	0.98	11 900	0.360
26/VI 1956	75%	6 000 000	1.0	11 250	0.342
3/VII 1956	75%	6 000 000	1.0	12 400	0.204
Average	75%	6 050 000	—	11 825	0.287
10/VII Date of operation when the Klementsievich – Heidenhain pouch was made					
21/VIII 1956	69%	5 160 000	1.08	17 000	0.128
29/VIII 1956	72%	5 900 000	0.97	14 625	0.144
5/IX 1956	72%	5 600 000	1.03	14 125	0.144
13/IX 1956	65%	5 000 000	1.04	14 250	0.07
17/IX 1956	58%	5 000 000	0.90	18 250	0.128
27/IX 1956	60%	4 060 000	1.20	18 750	0.022
2/X 1956	63%	4 080 000	1.09	12 500	0.022
10/X 1956	58%	5 360 000	0.87	14 350	0.072
17/X 1956	56%	4 760 000	0.95	17 000	0.072
Average	63.7%	5 057 000		15 650	0.089
23/X 1956					[1]
26/X 1956	60%	4 480 000	1.06		0.033[1]
30 X 1956	60%	4 600 000	1.04	16 250	0.072[1]
2/XI 1956					[1]
5/XI 1956	61%	5 300 000	0.90		0.264[1]
9/XI 1956	58%	5 080 000	0.90	18 750	0.233[1]
12/XI 1956					[1]
13/XI 1956	60%	4 400 000	1.09	18 750	0.233[1]
16/XI 1956					[1]
19/XI 1956	60%	5 000 000	0.96	17 300	0.072[1]
22/XI 1956	58%	4 400 000	1.05		0.072[1]
Average	59.5%	4 750 000		17 636	0.139
27/XI 1956	57%	4 400 000	1.03		
7/XII 1956	50%	4 000 000	1.0		

[1] Intramuscular injection of 15 γ of vitamin B_{12}.

The decrease in the vitamin B_{12} level in this animal is the result of disease which produces alterations in metabolism, these processes interfering with storage and utilization of the vitamin B_{12}.

Summarizing, our experiments show that partial gastric denervation in dogs leads after 40-60 days to a slight hyperchromic anemia not accompanied by a drop of the blood level of the vitamin B_{12}, this latter phenomenon being seen in two experimental dogs. This proves that the anemia in these cases is not due to disturbances in the absorption of the extrinsic factor.

This is corroborated also by the fact that intramuscular injection into the animals of vitamin B_{12} does not restore the hemogram completely.

SUMMARY

Anemia of the hyperchromic type appears in dogs 40-60 days after partial denervation of the stomach (formation of the small stomach pouch by Klementsievich-Heidenhain's method). It is not connected with decrease of vitamin B_{12} in the blood. Intramuscular introduction of vitamin B_{12} does not normalize the blood completely. These data permit the assumption that in this case development of anemia does not depend on disturbance of absorption of vitamin B_{12} introduced externally.

LITERATURE CITED

[1] V. N. Bukin, L. Ia. Areshkina and L. S. Kutseva, Biokhimiia, XIX, 6, 713-720 (1954).

[2] R. A. Durinian, Experimental Analysis of Anemias Developing After Denervation of Some Internal Organs,* Dissertation, Moscow, 1957.

[3] E. L. Kan, Materials for Studying the Influence of the Nervous System Upon the Composition of the Blood,* Dissertation, Leningrad, 1953.

[4] M. A. Kalnyn', The Effect of Spleen Denervation Upon Hemopoiesis* Candidate's Dissertation, Riga, 1955.

[5] I. P. Kikhtsier, B. M. Braginskii and V. N. Gershanovich, in: Vitamin B_{12} and its Clinical Applications,* Moscow, 1956, pp. 164-175.

[6] Iu. L. Milevskaia, ibid, pp. 146-152.

[7] O. M. Preger, Transactions Third Pavlovian Conference at Tomsk Medical Institute,* Tomsk, 1953, pp. 58-61.

[8] O. M. Preger, Transactions Fourth Pavlovian Conference at Tomsk Medical Institute,* 1954, 67-72.

[9] N. A. Fedorov, Contemporary Problems of Hematology and Blood Transfusions,* Moscow, 1955, section 31, pp. 75-77.

[10] N. A. Fedorov, A. M. Namiatysheva and A. M. Kakhetelidze, Problemy Hematol., I, 1, 10-16 (1956).

[11] V. N. Chernigovskii and A. Ia. Iaroshevskii, Problems of Nervous Regulation of the Blood System,* Moscow, 1953.

[12] S. I. Iakovlev, The Role and Significance of the Nervous System and B-Group Vitamins in Erythropoiesis* Dissertation, Leningrad, 1955.

* In Russian.

THE INFLUENCE OF THE NERVOUS SYSTEM ON THE COMPOSITION OF THE BLOOD

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At the basis of the contacts formed by an organism with its environment a considerable role is played by residual reactions of the nervous system. Academician A. D. Speranskii has demonstrated the possibility of reproducing pathological processes using the principle of trace reactions. He showed [9] that organs of the hemapoietic system are well innervated. This made possible a very important hypothesis concerning the influence of the nervous system on the blood-forming organs. The studies of A. D. Speranskii and others made it important to analyze the residual reactions in the nervous system during some forms of anemia.

The present study is an attempt to clarify the role played by the nervous system in the development and changes produced in the blood by phenylhydrazine poisoning.

We based this study on the statement that this chemical blood-destroying agent has the capacity to "produce a special form of nervous stimulation." Residual reactions during regeneration of the red blood have been observed by a whole series of workers [1, 2, 4, 8].

EXPERIMENTAL METHODS

In our studies the basic pathological process was produced by the introduction of an acid salt of phenylhydrazine, 0.5-1 ml of a 2% solution per 1 kg wt of animal being used. The phenylhydrazine was continued until the appearance of a pronounced anemia after which the blood was studied until the moment of normalization.

It became important to determine whether the blood picture characteristic for phenylhydrazine poisoning could be reproduced by using a new stimulant of the nervous system. As such a factor, we chose "towing." As is known, this method was suggested by A. D. Speranskii in 1935; with the aid of this procedure he was able to produce relapses of previously existing nerve dystrophic processes.

In our studies the first changes in the blood we investigated were those occurring after the infliction of trauma to the nervous system. To do this, we took 5 dogs and subjected them to "towing." When such experiments were performed, changes were observed both in the white and the red blood cell counts.

In the majority of instances, within 30 minutes of beginning the "towing," there was observed a small but constant rise in the hemoglobin, fluctuating between 3 and 10% and averaging 5%. There was observed a corresponding change in the r.b.c. count. By the end of the first day the hemoglobin and the erythrocyte number returned to original levels.

Similar alterations were seen in the leucocytes. Within 15 minutes the leucocyte count rose to 14,420, while within 15 more minutes it reached 20,800 per cc. By the 2nd day the count fell. The leucocytic reaction ended by the 3rd to 5th day, the original levels being restored. "Towing" also provoked changes in the number of reticulocytes (Fig. 1).

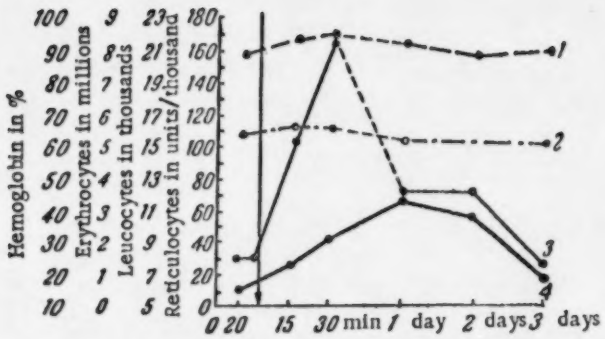

Fig. 1. Changes in some of the hematological indicators produced by "towing" (average of 5 experiments).
1) Hemoglobin; 2) erythrocytes; 3) leucocytes; 4) reticulocytes.
↓ "towing."

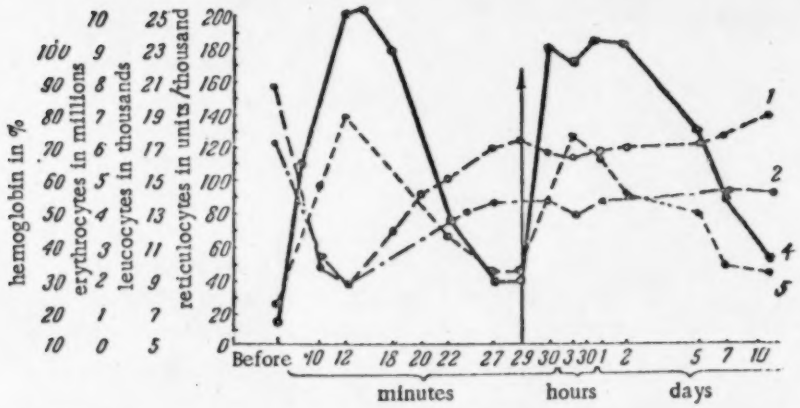

Fig. 2. Changes in some of the hematological indicators after "towing" (average of 8 experiments).
1) Hemoglobin; 2) erythrocytes; 3) leucocytes; 4) reticulocytes. ↑ "towing."

When examining blood smears, we observed that within 30 minutes after "towing," in spite of the leucocytosis, the leucocytic picture remained unchanged. This led to the conclusion that the number of leucocytes grew now because of new formation but as a result of blood redistribution [3, 10, 7].

The results obtained after "towing" gave us a basis of comparison for our second basic series of experiments.

In our experiments the hemotoxic anemia resulting from the phenylhydrazine poisoning usually developed by the 28th to 35th day. By that time the hemoglobin usually dropped to 27-29% (Fig. 2).

In all instances the greatest rapidity in the decrease in hemoglobin was noted in the first 5-7 days, after which the rapidity of the fall of the hemoglobin slackened somewhat.

Corresponding changes in the r.b.c. counts paralleled the hemoglobin fluctuations (Fig. 2). It is important to note that by the second or third day after the poisoning the regenerative capacity of the bone marrow became increased. In the peripheral blood there appeared normoblasts, which then regularly increased in number. At the same time there was a sharp rise in the reticulocyte count.

With such an experimental procedure there is observed a gradual increase in hemoglobin and the erythrocyte count. Usually, 10-12 days and more often not until 15-17 days after stopping the administration of the phenyl-

hydrazine, the animals emerged from their anemic state. By that time in some instances, the indicators have not all returned to base level and the hemoglobin content may be 10-12% below the original reading. However, the reticulocyte count dropped quickly and remained but little above base level (Fig. 2).

As far as the leucocyte counts were concerned, phenylhydrazine poisoning produced a pronounced leucocytosis with a shift to the left. In the peripheral blood, starting with 3rd to 9th days, there was a rise of up to 7-9% in the polys and up to 3-4% in juvenile neutrophils.

After the cessation of the poisoning, the leucocyte count dropped, reaching about base level.

Thus, after a lapse of 39 to 46 days following the poisoning of the animals with phenylhydrazine, there ensued a complete restoration of the hematological indicators. This gave us the opportunity to administer the "second blow" by means of "towing." After the "towing" we observed the entire course of the changes previously noted after the phenylhydrazine poisoning. There was a difference in that the maximal reticulocyte count did not reach the dimensions attained after the phenylhydrazine poisoning. However, when compared with the control "towing," there were both qualitative and quantitative differences. "Towing" alone raised the reticulocyte count 4.1-5.8% while "towing" after a preliminary background of phenylhydrazine poisoning caused a reticulocytosis of 17.8-18.2%. Thus, "towing" is shown to be a stimulant which, to a certain extent, can reproduce the blood picture of phenylhydrazine poisoning.

The experiments just described demonstrate that blood changes can be produced which resemble the picture of phenylhydrazine poisoning. The results we have obtained are interpreted by us as being the consequence of the nervous system retaining traces of previous stimuli.

Our experimental data seem to help explain up to a certain point some of the relapses seen during blood ailments as observed in the clinic. Not infrequently, a nonspecific stimulant such as grippe or psychic trauma is capable of causing a relapse in such pathological processes as pernicious anemia or chlorosis.

SUMMARY

"Followed-up - after-discharge reactions" were studied in the nervous system of laboratory animals in experimental anemia induced by phenylhydrazine. The method of the "second blow" with "towing" (Speranskii) was employed. It was possible to reproduce, to a certain degree, the blood picture characteristic of phenylhydrazine poisoning at the time when the blood picture almost reached its initial stage. It could particularly be reproduced in the line of the changes of processes of regeneration.

The data which was thus obtained gave theoretical foundation to these phenomena, which are sometimes met in clinical practice as relapses of certain diseases of the blood system.

LITERATURE CITED

[1] V. G. Vogarlik, Transactions of Tomsk Medical Institute. Tomsk 1937, section 1/4, pp. 1-68.

[2] D. I. Goldberg, Hematological Sketches (Blood Formation and the Nervous System)* Tomsk, 1952.

[3] A. Ia. Gubergrits, Autonomic Regulation of the Leucocytes,* Kiev, 1941.

[4] P. Ia. Mytnik, Arkh. Biol. Nauk XLV, Section 2, 103-122, 1937.

[5] I. P. Pavlov, 20-year Experience of Objective Study of the Response of the Higher Nervous System in Animals,* Moscow-Leningrad, 1938.

[6] A. D. Speranskii, Elements of Medical Theory,* Moscow, 1935.

[7] A. Iu. Tilis, Med. Zhur. 1941, Section 1, pp. 183-192.

[8] A. Iu. Tilis and Kh. Z. Liubetsky, Biull. Eksptl. Biol. i Med. No. 6, 52-54, 1953.

[9] V. N. Chernigovskii, Practical Problems of Blood Transfusion,* Moscow, 5-21, 1952.

[10] V. N. Chernigovskii and A. Ia. Iaroshevskii, Problems of the Nervous Regulation of the Hematopoietic System,* Moscow, 1953.

* In Russian.

ADRENALIN SECRETION AFTER SUPRAPLEURAL NOVOCAIN BLOCK OF THE SYMPATHETIC NERVES

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Assistant professor of the Kazan Veterinary Institute, V. V. Mosin, developed a method of suprapleural novocain block of the abdominal nerves with the associated sympathetic trunks in horses, large-horned cattle, sheep, pigs, dogs, dark brown foxes, cats, rabbits and other animals. Bilateral, suprapleural novocain block of the abdominal nerves and the bordering sympathetic trunks is of considerable therapeutic value in such diseases of the abdominal cavity as peritonitis, gastroenteritis, metritis, cystitis, nephritis, etc.

Experiments in our laboratory have demonstrated marked changes in the activities of the stomach, pancreas and other organs that result from the influence of the novocain block using the method of V. V. Mosin. In part, A. I. Poliakov demonstrated a lowering of the blood sugar which occurs after the block and lasts for over 10 hours and even up to several days.

It seemed important to clarify the mechanism of the hypoglycemia which develops after the block of the abdominal nerves and the sympathetic trunks. This would enable us to understand the reason for the favorable influence of the block under pathological situations. On the supposition that the development of the hypoglycemia is connected with a diminished secretion of adrenalin, we decided to determine the normal amount of adrenalin in the blood and then the amount present after a bilateral novocain block.

Blood adrenalin was determined by the method of luminescent analysis with the S. V. Senkevich modification developed in the laboratory of the Kazan Medical Institute.

In order to develop fluorescence of adrenalin or its derivatives, the blood plasma was subjected to the rays of a mercury quartz arc. The intensity of the luminescence was measured with a photometer.

The experiments were done on dogs. The blood was drawn from the femoral artery with a syringe rinsed in a 2% solution of acid sodium citrate. The site of blood withdrawal was flushed with alcohol. In each dog for the first 5-6 days the normal content of adrenalin was dete mined and then, at various intervals after the V. V. Mosin block, the adrenalin content was determined again. The block was performed using a 0.25% solution of novocain and injecting 20 cc of this solution on each side.

EXPERIMENTAL RESULTS

The normal blood adrenalin is shown in Table 1.

The data as presented demonstrate that luminescent analysis will always demonstrate the presence of adrenalin or its derivatives (in the blood). Normally the quantity of adrenalin in the blood fluctuates only within rather narrow limits. Sex and weight of the animals do not seem to affect the adrenalin blood levels.

After the normal values were determined for the blood plasma of all the dogs, a V. V. Mosin block was instituted and then, at various intervals, the blood adrenalin levels were again determined. The results of these tests are presented in Table 2. In succeeding days all the dogs showed a return of the adrenalin levels to normal.

TABLE 1

Alterations in the Amount of Adrenalin Present in the Blood of Dogs Under Normal Conditions

Date of the determinations	Adrenalin content in γ/cc in the dogs
Dog No. 1, Tresor, male, weight 8 kg	
21/VI	1.4
22/VI	1.9
Dog No. 2, Volchok, male, weight 8 kg	
20/VI	1.26
23/VI	1.5
Dog No. 3, Damka, female, weight 12 kg	
21/VI	1.2
22/VI	1.5
22/VI	1.1
Dog No. 4, Zhuchka, female, weight 9 kg	
22/VI	1.13
23/VI	1.1
Dog No. 5, Dzhilda, female, weight 18 kg	
12/VII	1.45
13/VII	1.1
14/VII	1.4
15/VII	1.2
16/VII	1.2
Dog No. 6, Orlik, male, weight 13 kg	
13/VII	1.45
14/VII	1.6
15/VII	1.8
Dog No. 7, Belka, female, weight 10 kg	
30/VII	1.3
31/VII	0.95
14/VIII	1.4
Dog No. 8, Sultan, male, weight 16 kg	
14/VIII	1.3
15/VIII	1.47

The adduced data show that within 2-3 hours after the block in the animals of the abdominal nerves and the associated sympathetic trunks, adrenalin and its products capable of fluorescing in the blood disappear. The fluorescing substances do not reappear until as late as 12 to 13 days after the block. The same results are obtained after a secondary block which was performed on dogs No. 5 and 6. In dog No. 5 fluorescent substances reappeared after the 12th day following the secondary block while in dog No. 6 they reappeared on the 13th day.

Thus, the novocain block of the absominal nerves interrupts impulses going to the suprarenals and their medullary substance thus producing profound alterations in the formation of adrenalin, the effects outlasting by far the actual action of the novocain itself. V. V. Mosin is of the opinion that his procedure produces anesthesia of the abdominal organs for only 2 to 5 hours. Apparently, then, the conductivity of the abdominal nerves is

interrupted for only a short time. However, the effects of the block persist much longer. According to the unpublished observations in our laboratory of Berestov, Lisov and Kostin, the block disturbs the actions of the stomach and pancreas for from 2 to 13 days.

It is evident that the adrenalin-forming function of the chromaffin tissues is also upset for a long time.

TABLE 2

Alterations in the Amount of Adrenalin Present in the Blood of Dogs Following Bilateral Novocain Block

Time interval	adrenalin content in the dogs expressed in γ/cc							
	№ 1	№ 2	№ 3	№ 4	№ 5	№ 6	№ 7	№ 8
block performed	6/24	6/24	6/25	6/25	6/16	6/16	6/5	6/15
After 3 hours. . . .	0	0	0	0	0	0		
" 4 "	—	—	—	—	0	0	0	0
" 48 " . . .	—	—	—	—	0	0	0	
" 3 days . . .	0	0	0	0	0	0		0
" 4 " . . .	—	—	—	—	0			
" 5 " . . .	—	—	—	—	0	0		0
" 6 " . . .	—	—	—	—	0	0		
" 7 " . . .	—	—	—	—	0		0	
" 8 " . . .	—	—	—	—	0	0		
" 9 " . . .	—	—	—	—	—	—	—	0
" 10 " . . .	—	—	—	—	0	0		0
" 11 " . . .	—	—	—	—	0	1.09	0	0
" 12 " . . .	—	—	—	—	0	1.0	0	0.95
" 13 " . . .	—	—	—	—	1.04	1.4	1.6	1.3

However, we cannot be certain that the disappearance of fluorescing substances from the blood must mean that the novocain block stops the formation of adrenalin. It may be supposed that under the influence of cessation of afferent impulses to the chromaffin tissues the chemistry of adrenalin formation becomes so altered that fluorescent substances no longer become the end product of adrenalin metabolism.

The problem needs further investigation. We believe that we have established the fact that fluorescent substances disappear from the blood as a result of novocain block of the abdominal nerves and the sympathetic nerves by the V. V. Mosin method and that this fact must be taken into consideration when the mechanism of the action of this block is examined further and practical therapeutic conclusions are being drawn.

Thus, the suprapleural novocain block of the abdominal nerves and associated sympathetic trunks by the V. V. Mosin method produces a disappearance from the blood of the fluorescing products of adrenalin metabolism. The action of the block persists for 12 to 14 days at the end of which time these fluorescing substances reappear in the blood. This effect of the novocain block must be associated with the cessation of impulses going over the abdominal nerves to the suprarenals.

SUMMARY

Experiments were performed on dogs to reveal the mechanism of hypoglycemia which takes place in novocain block of the abdominal nerves and sympathetic trunks. The method of luminiscent analysis was used.

It was established that a stable disappearance of the products of adrenalin disintegration takes place in the blood and plasma even 2-3 hours following suprapleural novocain block of the abdominal nerves and the sympathetic trunks by Mosin's method. The function of adrenalin production is reestablished on the 12th-13th day after the block.

LITERATURE CITED

[1] V. V. Mosin, Novocain Block of the Abdominal Nerves – A Method for Producing a Protective Effect Upon the Nervous System in the Presence of Peritonitis and Inflammation of Abdominal Organs,* Dissertation, Kazan, 1951.

[2] S. V. Senkevich, A Method of Luminescent Analysis of Blood Adrenalin and Tissue Sympathin,* Dissertation, Kazan, 1953.

* In Russian.

BIOCHEMISTRY AND BIOPHYSICS

DISTRIBUTION OF NUCLEIC ACIDS IN THE CELL NUCLEI OF NORMAL LIVER AND TRANSPLANTED MOUSE HEPATOMA

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I. B. Zbarskii and S. S. Debov [1-3] have shown that three protein fractions can be obtained from the cell nuclei isolated from normal tissues and malignant tumors in man; these authors called the three fractions "nucleoprotein," "acid protein" and "residual protein."

The ratio of these fractions in the cell nuclei of malignant tumors differs from the normal in the low content of nucleoprotein and acid protein and a sharp increase (5-10-fold) in residual protein content [1, 2].

These protein fractions from cell nuclei vary in their amino acid composition [3]. With respect to their content of nucleic acids, the authors only give data concerning the phosphorus content and indicate qualitative detection of desoxyribonucleic acid (DNA) in the nucleoprotein fraction only [1, 3].

The present work is concerned with investigation of the DNA and ribonucleic acid (RNA) content of the cell nuclei and nuclear protein fractions in normal liver and transplanted mouse hepatoma.

EXPERIMENTAL METHODS

Mice of the strain "S_3NA" were inoculated subcutaneously with hepatoma. They were sacrificed on the 18th-20th day after the inoculation and the tumor was removed for examination.

Cell nuclei were isolated from 8-10 g liver or tumor tissue obtained from several animals.

The cell nuclei were isolated in a neutral medium by the method of A. Dounce [7] or by the Zbarskii and Perevoshchikova modification of this method [4] and then fractionated by the procedure described earlier [1].

The content of the nuclear fractions was expressed in percentage nitrogen of the given fraction with respect to the total nuclear nitrogen.

The isolation and separation of nucleic acids was carried out according to G. Schmidt and S. I. Thannhauser [12] in the H. W. Kosterlitz [10] modification. The amount of DNA was determined in terms of desoxypentose using the colorimetric method of I. M. Webb and H. B. Levy [13] and parallelly in terms of phosphorus; RNA was determined by way of phosphorus. Nitrogen estimations on individual samples were performed by the Kjeldahl micromethod; phosphorus was estimated by the C. Fiske and G. Subbarow [9] method.

Since it is extremely difficult to determine the dry weight and to express results as percentages of dry weight when cell nuclei and nuclear fractions are isolated from small amounts of material under investigation, the calculations of nucleic acid content of such material were made in percentages of the total nitrogen content multiplied by 6.25 ("total protein").

The results obtained (see Table) indicate that the DNA content of cell nuclei in normal mouse liver is on the average 12.3 $\pm$ 0.7% of their total protein; this corresponds to data in the literature [5, 6, 8].

In the case of the cell nuclei isolated from mouse hepatoma the DNA content is equal to 3.8 $\pm$ 0.2% of the total protein, i.e., it is one-third of that in the nuclei of normal liver; this can evidently be explained by the raised content of the residual protein fraction in the nuclei of the tumor cells.

DNA and RNA Content of Cell Nuclei and Nuclear Fractions in Normal Mouse Liver and Transplanted Mouse Hepatoma

No. of expt.	Nuclei			Nucleoprotein		Acid protein	
	In % of $N_{x6.25}$		RNA / DNA	in % of total protein in the nuclei		in % of total protein in the nuclei	
	DNA	RNA		DNA	RNA	DNA	RNA
			Mouse liver				
1	10.4	1.1	0.10	98.0	61.1	1.6	37.0
2	14.7	1.0	0.06	98.2	67.0	1.0	31.7
3	10.7	0.7	0.06	98.0	61.3	1.5	38.1
4	14.0	1.2	0.08	97.4	70.1	1.2	28.8
5	12.1	1.3	0.10	95.0	55.0	3.0	45.0
6	12.0	1.4	0.11	96.0	58.0	2.8	40.3
	Average 12.3±0.7	1.1±0.1		97.1±0.5	62.0±0.3	1.8±0.3	36.8±0.8
			Mouse hepatoma				
7	4.1	0.6	0.14	95.0	51.3	2.5	46.1
8	3.2	0.5	0.15	95.7	53.0	4.3	47.0
9	4.0	0.8	0.2	91.2	52.3	4.1	45.3
10	4.6	0.8	0.17	90.0	58.0	4.4	42.0
11	3.6	0.6	0.17	94.3	55.0	4.0	43.3
12	3.8	0.4	0.10	98.2	54.3	1.7	45.7
	Average 3.8±0.2	0.6±0.06		93.0±1.3	53.9±0.9	3.5±0.4	44.9±0.8

The RNA content of cell nuclei in the liver approaches the literature data [6, 8] and is about 1% of their total protein. The amount of RNA in the cell nuclei of hepatoma is nearly half that of normal liver nuclei and is, on the average, 0.6% of their total protein. However, the ratio of RNA and DNA in hepatoma is nearly double that of normal liver nuclei.

The nucleoprotein fraction extracted by 1 M NaCl solution contains almost all (90-98.2%) the cell nuclei DNA, whether the nuclei are isolated from normal liver or transplanted mouse hepatoma. These results confirm the data reported by Mirsky and collaborators [11] who stated that almost all the DNA could be extracted from nuclei by means of 1 M NaCl solution.

Nuclear RNA is found in the nucleoprotein fraction and in acid protein, its amount in the nucleoprotein fraction apparently depending to a considerable extent on admixture of cytoplasm; thus, the RNA content in the nucleoprotein fraction of less thoroughly purified nuclei with early agglutination is considerably raised.

No nucleic acids are found in the residual protein.

The results obtained thus make it possible to characterize more precisely the nuclear fractions of normal liver and transplanted hepatoma from the point of view of their nucleic acid content. It is probable that the acid protein fraction can in some measure be regarded as a ribonucleoprotein fraction, while residual protein evidently does not form complexes with nucleic acids.

No significant differences are found in the distribution of nucleic acids in the nuclear fractions of normal and tumor nuclei; the RNA—DNA ratio, however, is higher in tumor nuclei than in the normal.

SUMMARY

It was shown that desoxyribonucleic acid (DNA) constituted 10-14% of dry substance of cellular nuclei in normal liver and about 3-4% of the nuclei of mice hepatoma. Practically all DNA (90-98%) is found in nucleo-

protein fraction in both cases. Ribonucleic acid (RNA) constitutes 0.4-1.4% of dry substance of the cellular nuclei in normal liver as well as in transplanted hepatoma in mice. However, the ratio of RNA to DNA in the nuclei of hepatoma is greater than normal. Nuclear RNA is found both in the nucleoprotein fraction and in acid protein. Its quantity evidently depends to a great extent on the admixture of cytoplasm. No nucleic acids are found in the residual protein.

LITERATURE CITED

[1] S. S. Debov, Biokhimiia, 16, No. 4, 314-320 (1951).

[2] I. B. Zbarskii and S. S. Debov, Doklady Akad. Nauk SSSR, 62, No. 6, 795-798 (1948).

[3] I. B. Zbarskii and S. S. Debov, Biokhimiia, 16, No. 5, 390-395 (1951).

[4] I. B. Zbarskii and K. A. Perevoshchikova, Biokhimiia, 16, No. 2, 112-124 (1951).

[5] A. L. Dounce, In the book: Modern Problems of Cytology,* Moscow, 1955, pp. 51-71.

[6] A. W. Pollister, In the book: Modern Problems of Cytology,* Moscow, 1955, pp. 91-103.

[7] A. L. Dounce, Journ. Biol. Chem. 1943, Vol. 147, No. 3, pp. 685-698.

[8] A. L. Dounce, The Nucleic Acids, 1955, Vol. 2, p. 97.

[9] Ch. Fiske and I. Subbarow, J. Biol. Chem. 1925, Vol. 66, pp. 375-400.

[10] H. W. Kosterlitz, J. Physiol., 1947, Vol. 106, No. 2, pp. 194-210.

[11] A. E. Mirsky and A. W. Pollister, J. Gen. Physiol., 1946, Vol. 30, No. 2, pp. 117-147.

[12] G. Schmidt and S. I. Thannhauser, J. Biol. Chem., 1945, Vol. 161, No. 1, pp. 83-89.

[13] I. M. Webb and H. B. Levy, J. Biol. Chem., 1955, Vol. 213, No. 1, pp. 107-117.

* Russian translation.

VARIATIONS IN THE BLOOD BROMINE LEVEL DURING SENSITIZATION, ANAPHYLACTIC SHOCK AND THE POSTSHOCK PERIOD

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The interrelationship of the blood bromine level and the character of the relation between the main nervous processes – excitation and inhibition – in the cerebral cortex was first shown by I. P. Pavlov's collaborators L. M. Georgievskaia, M. K. Petrova and M. A. Usievich [4, 7]. Subsequently it became evident that dogs with different types of nervous systems exhibited definite fluctuations in the blood bromine level, characteristic for the given type [2], that interference of the food and defense reactions caused a sharp disturbance of stability in this level [1] and that radium irradiation of the animal's head was accompanied by a decrease in bromine concentration in the blood [6].

The present work is concerned with the determination of dynamics of the blood bromine level in dogs under normal conditions and in various stages of the anaphylactic state, starting from the moment of protein sensitization and finishing with the postshock period.

EXPERIMENTAL METHODS

Bromine concentration in the blood was determined by Greenberg's micromethod [9], based on van der Meilen's principle and consisting of oxidation of bromides to bromates with subsequent iodometric titration. In carrying out long-term experiments on the dynamics of fluctuation in the blood bromine level it is essential that the experimental animals be maintained on a constant diet, since different foodstuffs have extremely variable bromine content. Moreover, it is essential to take into account the definite interrelations of bromine and chlorine in the organism. Cases have been described in which a sharp rise in blood bromine was noted in patients who had been using a great deal of common salt in their food [10].

The animals were sensitized with normal horse serum according to the generally accepted method. The serum was administered on 3 successive days in doses of 0.2 ml per 1 kg body weight (subcutaneously on 2 days, intravenously on the 3rd day). Anaphylactic shock was produced on the 20th-23rd day from the moment of sensitization by means of a booster dose of specific serum (2 ml per 1 kg body weight).

EXPERIMENTAL RESULTS

Table 1 shows the data of 11 experiments, giving the extreme fluctuations in the blood bromine level before and during sensitization. In most dogs the fluctuations in the blood bromine content were within the limits of 0.1 mg%, but in isolated cases these fluctuations amounted to 0.15-0.26 mg%. Such variability among dogs with respect to blood bromine content had been noted previously (M. F. Vasil'ev [2]).

During sensitization, beginning with the 8th day after administration of the first sensitizing dose, the majority of cases showed a tendency to decrease blood bromine concentration. In one dog (protocol 5) an increase of blood bromine level from 1.292 to 1.596 mg% was observed during the period of sensitization. The extreme fluctuations in the blood bromine level observed during the different days of sensitization exceeded considerably those limits which were characteristic for the dogs prior to sensitization. This was particularly pronounced at the height of anaphylactic state, i.e., during the days of the most violent reaction to repeated injection of the antigen. It is

TABLE 1

Extreme Fluctuations of Blood Bromine Levels in Dogs in the Normal State and During Sensitization

No. of experiment	Fluctuations of bromine levels in the normal (in mg %)	Fluctuations of blood bromine levels on successive days of sensitization (in mg %)					
		1–4	5–7	8–12	13–16	17–19	20–23
1	0.813 0.912	0.912 0.912	0.760 0.836	0.988 1.140	0.646 0.684	0.342 0.380	0.372 0.752
2	1.064 1.140	1.064 1.064	0.676 0.684	0.646 0.760	0.646 0.691	0.364 0.524	0.121 0.380
3	0.676 0.760	0.304 0.380	0.433 0.433	— —	— —	0.281 0.357	0.235 0.934
4	0.889 0.988	0.912 0.919	0.380 0.836	— —	— —	0.836 0.912	0.630 0.874
5	1.064 1.140	1.140 1.292	0.836 1.444	1.444 1.520	0.988 0.988	0.912 1.596	0.836 1.064
6	0.912 1.064	0.836 0.836	0.737 0.737	— —	— —	0.380 0.532	0.380 0.942
7	0.304 0.326	0.228 0.456	0.205 0.205	0.152 0.319	0.190 0.205	0.190 0.448	0.152 0.471
8	0.532 0.653	0.532 0.585	0.380 0.380	0.228 0.304	0.167 0.190	0.144 1.144	0.136 0.448
9	0.372 0.418	0.372 0.380	0.167 0.304	0.167 0.228	0.205 0.304	0.152 0.212	0.722 0.828
10	0.190 0.342	0.197 0.212	0.152 0.190	0.114 0.152	0.076 0.136	0.091 0.121	0.091 0.152
11	0.471 0.737	0.478 0.684	0.311 0.494	0.273 0.357	0.250 0.305	0.205 0.372	1.197 0.668

TABLE 2

Fluctuations of Blood Bromine Level in Dogs During Anaphylactic Shock

No. of experiment	Initial blood bromine level (in mg %)	Time in minutes from the booster administration of serum and the corresponding blood bromine levels (in mg %)						Outcome of shock
		2–5	10–15	20	30	40–50	60–180	
1	0.76	3.42	3.42	5.32	--	2.40	0.13	Alive
2	0.38	3.31	4.25	3.72	0.11	—	—	Died
3	0.93	0.53	0.47	0.22	—	—	—	"
4	0.63	3.61	3.30	3.34	—	2.88	3.26	Alive
5	0.83	2.88	1.59	—	2.28	2.35	2.50	"
6	0.88	2.58	2.20	0.15	—	—	—	Died
7	0.47	1.87	1.45	0.16	0.77	—	0.54	Alive
8	0.42	5.09	0.72	1.25	—	1.14	0.72	"
9	0.82	2.84	2.55	1.59	0.06	—	—	Died
10	0.15	0.49	2.09	0.07	—	—	—	Died
11	0.66	2.23	0.17	—	—	—	—	Died
Control experiments								
12	0.68	0.60	0.63	0.65	0.64	0.65	0.66	No reaction
13	0.32	0.31	0.31	0.32	0.30	0.34	—	

[1] Serum, 2 ml per 1 kg body weight, administered for the first time.

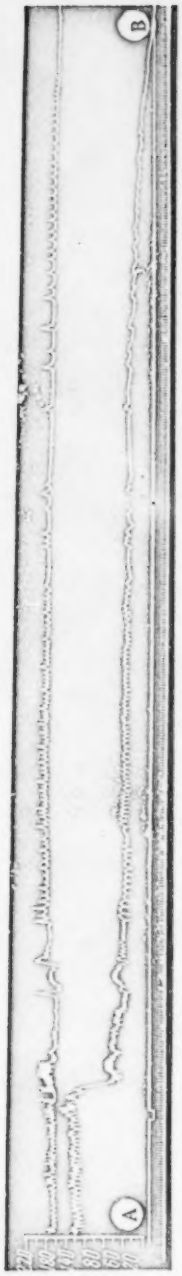
known that during these days changes of excitability and functional mobility of peripheral neuromuscular formations are particularly marked [5] as are disturbances of the dynamics of the main nervous processes – excitation and inhibition – at the highest levels of the central nervous system [3, 8]. It could be taken, in connection with the data cited, that the observed changes in blood bromine concentration could be determined by changes in the functional state of the nervous system in protein sensitization.

The most marked and consistent changes in blood bromine content were observed during anaphylactic shock. Phasic disturbances of nervous activity were, as a rule, accompanied by biphasic fluctuations in the blood bromine level. As can be seen from Table 2, if there was accummulation of bromine in the blood, sometimes reaching high values (2.88-3.61-5.09 mg%), during the first few minutes after the booster injection of serum then subsequently, as the torpid phase of shock developed and deepened, the blood bromine level passed to the second phase – the phase of gradual diminution.

It must be emphasized that in all cases of anaphylactic shock with fatal outcome, hypobromemia was found a few minutes before death, while in cases of survival the blood bromine level during anaphylactic shock remained raised, to a greater or lesser extent, as compared with the initial level; this continued for $1\frac{1}{2}$-2 hours after administration of the booster dose of serum (see Fig).

During the postshock period no consistent fluctuations in the blood bromine level could be observed: in 2 dogs hypobromemia was still noted 20 days later, while in 3 others the blood bromine level was similar to the initial level within the first 24 hours after shock.

The present material indicates that the appearance and development of the anaphylactic state is accompanied by changes in blood bromine content. Phasic disturbances of nervous activity during anaphylactic shock evidently determined the biphasic fluctuations of the blood bromine level. The findings concerning the biphasic changes in the blood bromine content after the booster injection of serum can be tentatively explained on the assumption that at the beginning of shock bromine passes from most of the tissues into the blood and is then transferred to other organs, including the central nervous system where bromine is known to favor enhancement and concentration of inhibitory processes. From this point of view, the hypobromemia observed during the first few minutes following the booster administration of serum may be regarded as a defensive reaction of the organism against the "excessive stimulus," viz. antigen.



Changes in blood pressure and respiration after booster injection of serum (Experiment 11). Records (from above down): respiratory movements, blood pressure in the femoral artery, base line and signal of serum injection. Time marker (2 seconds). A) Normal, B) 15 minutes after repeat injection.

SUMMARY

In the majority of cases of protein sensitization in dogs, beginning from the 8th to the 12th day following the introduction of the first sensitizing dose, there was a general tendency of the blood bromine level to decrease. Besides, from the 18th to the 22nd day – the last days of sensitization – there was relative instability of the blood bromine level. Variations of the latter during anaphylactic shock were of a biphasic character, i.e., hyperbromemia at the onset of shock was replaced by gradual reduction of the bromine level which sometimes

fell to marked hypobromemia. The latter was especially pronounced in animals which died within the first hour after the introduction of the booster of the serum.

No regular deviations in the blood bromine content were revealed in the postshock period.

LITERATURE CITED

[1] L. B. Andreev, Trudy Inst. fiziol. im. I. P. Pavlova, Leningrad, III, 607-611 (1954).

[2] M. F. Vasil'ev, Trudy fiziol. laboratorii im. akad. I. P. Pavlova, Leningrad, XIV, 83-87 (1948).

[3] O. D. Gaske, In the book: 16th Conference on the Problems of Higher Nervous Activity,* Moscow-Leningrad, 1953, pp. 58-60.

[4] L. M. Georgievskaia and M. A. Usievich, Fiziol. Zhur. SSSR, 18, 166-179 (1935).

[5] M. I. Rafiki, Vestn. Lenigr. Universiteta, Leningrad, 9, 93-111 (1950).

[6] F. I. Rivosh, Vestnik Rentgenol. i Radiol., 20, 83-89 (1938).

[7] M. A. Usievich and M. K. Petrova, In the book: Physiology of Higher Nervous Activity,* Moscow, 1953, pp. 182-193.

[8] L. E. Khozak, Zhur. Vysshei Nerv. Deiatel., III, 144-145 (1953).

[9] Greenberg, Journ. lab. clin. med., 1943, Vol. 28, pp. 779-786.

[10] Meier and Schlintz, Klin. Wschr. 1936, No. 11, pp. 1845-1847.

* In Russian.

THE EFFECT OF SODIUM TARTRONATE ON THE BODY WEIGHT AND ON THE FAT AND GLYCOGEN CONTENT OF MOUSE AND RAT LIVERS

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It is well known that one of the most important factors in the development of obesity is the acceleration of carbohydrate transformation into fat (S. M. Leites [2]). It was therefore of interest to attempt the discovery of such biological factors, that would be capable of inhibiting this process and could thus be utilized in the treatment of obesity. There are indications in the literature (Weston [4]) that the introduction of tartronic acid (or of its sodium salt) caused a depression of the respiratory quotient in rats which had been placed on a fat-free diet and which, consequently, actively converted carbohydrate into fat. The observation was interpreted by Wesson as an indication of the suppression by tartronate of carbohydrate to fat conversion.

The liver is the main organ in which the conversion of carbohydrate to fat takes place. In order to investigate the extent of tartronic acid effect on the above mentioned process, it was of interest to study the effect of tartronic acid administration on the glycogen to fat ratio in animals receiving excess carbohydrate.

EXPERIMENTAL METHODS

Rats and mice were placed on a standard mixed diet, supplemented with glucose in the milk over a period of 10 days; 2 g of glucose was given to each rat, and 0.5 g to each mouse, daily. During the same 10-day period a daily dose of sodium tartronate ranging from 0.025 to 0.09 g was administered as a 5% aqueous solution, orally to one group of animals, subcutaneously to others.

For the estimation of fat, liver powders dried to constant weight were extracted with dichloroethane (S. M. Leites and G. T. Pavlov [3]); glycogen was estimated by the micro-Pfluger technique, as modified by V. I. Goncharova [1]. Thirty-three treated and 29 control rats, and 12 each treated and control mice, were employed in the experiment.

EXPERIMENTAL RESULTS

As will be seen from the data presented in the Table, supplementation of the diet with sodium tartronate (NaT) over the 10-day period had no effect on the body weight or on the fat and glycogen content of the livers (1st series). Subcutaneous introduction of NaT, while having no effect on the fat and glycogen content of the livers, inhibited body weight increase in the group of experimental animals (2nd series). When the NaT dose was increased to 0.09, subcutaneously, there was a tendency in adult rats, apart from the inhibition of body weight increase, toward an increase in liver glycogen content, and a lower fat content (3rd series). It should be underlined that this effect was observed only with adult animals (weight range – 254 to 364 g); in growing rats (96-110 g) fat and glycogen content did not alter, even though the inhibition of increase in body weight was observed (4th series). A similar decrease in fat content and increase in glycogen content of the liver was observed in adult (body weight 31-33 g) mice on administration of 0.025 g of NaT subcutaneously (5th series).

The Effect of Sodium Tartronate Treatment on the Animal Weight and on Fat and Glycogen Content of the Livers (mean values)

Animal species and experimental series	Expt.							Control					
	number of animals	daily NaT dose	introduction route	weight (in g)			liver		number of animals	weight (in g)			liver	
				before the experiment	at the end of the 10-day period	difference	fat (g%)	glycogen (g%)		before the experiment	at the end of the 10-day period	difference	fat (g%)	glycogen (g%)
Rats														
1st series	11	0.025	Per os	161	166	+5	16.2 (13.5—20.5)[2]	2.49	12	160	165	+5	15.0 (11.5—18.0)	2.38
2nd series	10	0.025	Subcut.	171	192	+21	14.0	4.2	6	172	209	+37	14.0	4.1
3rd series	9	0.09	» »	307 (254—364)	294 (207—381)	—13	14.6 (12.0—15.5)	3.6	7	319 (256—367)	335 (244—396)	+16	16.3 (13.5—19.5)	2.4
4th series	3	0.075	» »	102	112	+10	16.3	3.8	4	105	136	+31	15.8	3.7
Mice														
5th series	12	0.025	» »	33	31	—2	16.9 (14.5—20.5)	1.46 (0.67—2.5)	12	31	30	—1	19.6 (17.0—23.0)	0.64 (0.28—1.2)

[1] Fat expressed on dry weight basis.

[2] Limits of variation shown in brackets.

Thus, the subcutaneous introduction of tartronic acid to adult rats and mice was followed by an increase in liver glycogen content and a parallel decrease in fat content; in the case of rats, there was also a decrease in body weight. These results may be interpreted as an indication that in adult animals tartronic acid effect may have been to inhibit the process of carbohydrate transformation into fat.

SUMMARY

The effect of sodium tartronate was studied in experiments on mice and rats on the basis of data on the inhibition of the transfer of carbohydrates into fats by tartronic acid presented by Wesson. Sodium tartronate was administered in doses of 0.025 to 0.09 g and its effect on body weight, fat and liver glycogen was studied. If 0.09 g of sodium tartronate is introduced subcutaneously to adult rats weighing from 254 to 364 g for 10 days, reduction of weight, increase of glycogen content and a certain decrease of fat content is noted in the liver. If 0.025 g of this substance is introduced subcutaneously to adult mice weighing 31 to 33 g, there is an increase of glycogen and decrease of fat in the liver. However, with mice there is no reduction of weight.

LITERATURE CITED

[1] V. I. Goncharova, Biull. Exptl. Biol. i Med. No. 7, 44-46 (1952).

[2] S. M. Leites: The Physiology and Pathology of Adipose Tissue,* Moscow 1954.

[3] S. M. Leites and G.T. Pavlov, Biull. Exptl. Biol. i Med. 25, No. 5, 374-378 (1948).

[4] Wesson, Endocrinology, No. 47, pp. 302-304, 1950.

* In Russian.

PHARMACOLOGY

THE COMBINED EFFECT OF CYTOCHROME SYSTEM POISONS AND POISONS INTERFERING WITH RESPIRATORY PHOSPHORYLATION ON THE TOLERANCE OF MICE TO LOWERING OF ATMOSPHERIC PRESSURE

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The present investigation was prompted by our observation which showed, unexpectedly, that sodium azide and potassium cyanide increased the tolerance of animals to lowering of atmospheric pressure.

It is known that sodium azide and potassium cyanide block the cytochrome system in the tissues. In recent years a number of investigations performed on isolated tissues [7, 8, 11 etc] showed that azide could interfere with the processes of conjugated phosphorylation; this interference can be effected in concentrations insufficient to impair utilization of oxygen mediated by the cytochrome system [4].

EXPERIMENTAL METHODS

The experiments were performed in a pressure chamber (capacity 5 liters); the animals used were adult white mice weighing from 16 to 22 g. The rate of decompression was equal to 1 minute in all experiments, the time spent by the animals in the pressure chamber was 10 minutes. The experiments were thus staged under conditions of acute and brief oxygen lack. In addition to the 2-3 experimental animals the same number of control animals from the same batch were placed in the pressure chamber. Sodium azide and potassium cyanide were administered subcutaneously in the form of 0.1-0.2% solutions in doses of 15 γ per 1 g body weight. It must be noted that the potassium cyanide available was partially decomposed and therefore the minimal lethal dose (20 γ per 1 g body weight) was approximately double that given in literature references. The doses of azide used did not produce any changes in the behavior of the animals, while administration of cyanide was accompanied, in a number of experiments, by limitation of the animals' motor activity.

EXPERIMENTAL RESULTS

Results of the first series of experiments are presented in Table 1.

TABLE 1

Effect of Sodium Azide and Potassium Cyanide on the Tolerance of Mice to Lowering of Atmospheric Pressure (220-230 mm Hg)

Doses in γ per 1 g		Interval between injection of poisons and placing in pressure chamber (in minutes)	Number of animals		Number of survivals out of 5
NaN_3	KCN		Total in experiment	Survived	
–	–	–	75	11	1
15	–	10	5	0	0
		25-45	30	25	4
		90	5	0	0
–	5	5	5	0	0
		10-35	25	20	4
		45-50	5	1	0

As can be seen from Table 1, both sodium azide and potassium cyanide not only did not diminish the tolerance of mice to lowering of atmospheric pressure, but on the contrary increased this tolerance. This effect became apparent in 5-20 minutes when azide was used and continued for not more than 1½ hours. When cyanide was used the increased tolerance to hypoxia became evident within 10 minutes but proved to be more short-lived, disappearing after 45 minutes. Smaller doses of azide did not affect the animals' tolerance to lowering of atmospheric pressure.

In order to discover whether this increase in tolerance was connected with blocking of the cytochrome system or other links in tissue respiration we made use of data contained in the thesis of Z. N. Ivanova (1939). According to these data, administration of azide in the dose corresponding to approximately 60% of the lethal one led to a sharp drop in the animals' tolerance to cyanide intoxication: the mice died from 50% of the lethal dose of cyanide. Such decreased tolerance persisted up to 48 hours, whereas decreased tolerance to azide following preliminary administration of cyanide disappeared with 30-80 minutes. Z. N. Ivanova concluded that the duration of cytochrome system block elicited by these two poisons was very dissimilar.

Our experiment was performed as follows. 20 mice were given 15 γ per 1 g body weight azide and after 24 hours half the mice were checked for tolerance to lowering of atmospheric pressure and the other half for tolerance to cyanides.

It was found, as should have been expected from the data of the first series of experiments, that the tolerance to lowered atmospheric pressure was no different in animals treated with sodium azide 24 hours previously from that in the control (unpoisoned) mice: in the control group one mouse out of 10 survived decompression of 250 mm Hg, while 2 out of 10 survived in the experimental group. At the same time, the animals' tolerance to cyanide proved to be sharply reduced: of 10 control mice (which received cyanide only in the dose 10 γ/g) 9 survived, whereas of 10 experimental mice (which were given azide the previous day and cyanide on the day of the experiment) only one survived. Smaller doses of azide (10 γ/g) did not reduce the tolerance to cyanides.

Thus, 24 hours after administration of azide it still decreases tolerance to cyanides sharply but has no effect on tolerance to lowering of atmospheric pressure. The results of these experiments give grounds for distinguishing two toxic effects of azide: a more prolonged one, discovered by Z. N. Ivanova and expressed in reduction of tolerance to cyanides and a more transient one expressed in increase of animal tolerance to lowering of atmospheric pressure. Both azide effects were observed when the same doses of the substance were used.

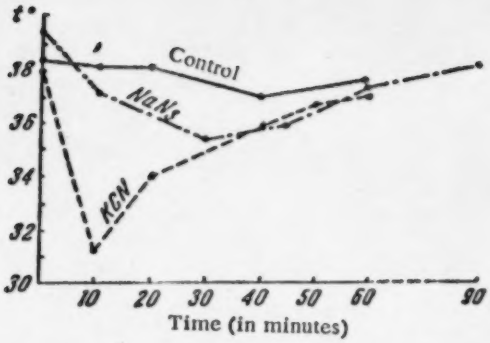
TABLE 2

Effect of Sodium Azide and Potassium Cyanide on the Tolerance of Mice to Lowering of Atmospheric Pressure (250 mm Hg) Weakened by Administration of Dinitrophenol

Substances administered	Number of animals		% survivals
	Total in experiment	Survived	
Control	30	17	57
Dinitrophenol	50	8	16
KCN and dinitrophenol	25	21	84
NaN_3 and dinitrophenol	25	24	96

It was postulated that the second azide effect, increase of tolerance to lowering of atmospheric pressure as shown by mice, could depend on lowering of body temperature under the influence of this substance. Increased tolerance of animals to hypoxia of various origins, including lowering of atmospheric pressure, associated with lowering of body temperature has been demonstrated by a number of authors [1, 2, 5, 6 etc].

To check this hypothesis, in a number of experiments the rectal temperature of mice was taken by means of a microthermometer before administration of azide and cyanide and after their administration prior to placing



Effect of sodium azide and potassium cyanide on the rectal temperature of mice.
Each curve represents mean data obtained from 5 mice.

the animals in the pressure chamber. Simultaneous temperature readings were taken from control animals. In addition, experiments were carried out on 15 mice (5 control, 5 given azide, 5 given cyanide in doses cited above) with determination of temperature at 10-minute intervals over a period of $1\frac{1}{2}$ hours (see Fig).

As can be seen from the graph, both azide and cyanide elicit definite lowering of body temperature whose onset and duration correspond to the time during which the tolerance of mice to lowering of atmospheric pressure is increased by these agents. However, although administration of cyanide led to a greater lowering of temperature it was not accompanied by greater increase in tolerance to reduction of atmospheric pressure (see Table 1).

Since azide and cyanide also impair conjugated phosphorylation (by depression of respiration) it was found essential to investigate the combined effect of these poisons and dinitrophenol (DNP) – a poison that impairs conjugated phosphorylation directly. Such an investigation was all the more pertinent since it was known that DNP lowered animal tolerance to hypoxia [3, 13].

This series of experiments was carried out both in and out of the pressure chamber. Azide potentiated the effects exerted by DNP under the latter conditions: under the influence of azide the tolerance of mice to dinitrophenol intoxication was reduced. Thus, when DNP alone was given in the dose 25-30 γ/g, 16 out of 25 mice survived (64%), while when the same dose of DNP was preceded by administration of 15 γ sodium azide 10 minutes earlier, only 6 out of 25 mice survived (24%).

Different results were obtained in the pressure chamber. Sodium azide and potassium cyanide were given in the same doses as in the preceding experiments: azide was given 35 minutes prior to decompression and cyanide 20 minutes prior to decompression. DNP was given subcutaneously 30 minutes before the beginning of decompression in doses of 5, 10 and 15 γ/g in the form of 0.05-0.1% solution (with addition of sodium bicarbonate to facilitate solution). It was found that azide and cyanide abolished the DNP-elicited reduction of tolerance to lowering of atmospheric pressure. The most definite results were obtained when DNP was given in the dose 5 γ/g (Table 2). On increasing the dose of DNP to 10 γ/g the preventive effect of azide was diminished and completely eliminated when 15 γ/g DNP was given.

The difference in results obtained in and out of the pressure chamber is doubtless connected with the fact that in the former case the doses of DNP were one-fifth to one-sixth of those used in the latter case: the toxic effects of considerably smaller doses of DNP become apparent in the pressure chamber and it is these particular effects that are suppressed by azide and cyanide.

References have appeared in recent years in the literature [9, 10, 12] showing that azide suppresses the ability of DNP to activate apyrase or ATP-ase. We are not aware of a similar effect being exerted by cyanide. Therefore, antagonism in the effects of DNP and sodium azide has also been established biochemically. The question of whether this antagonism can be identified with that discovered in our experiments is still not clear and needs further investigation.

SUMMARY

Sodium azide and potassium cyanide increase the tolerance of mature white mice to reduced atmospheric pressure. This effect takes place also in those cases in which the resistance of animals is decreased by the administration of dinitrophenol.

LITERATURE CITED

[1] V. S. Vail', Pediatriia, No. 5, 13-16 (1943).

[2] E. V. Gubler, Biull. Eksptl. Biol. i Med., 37, No. 2, 34-40 (1954).

[3] S. V. Osipova, Thesis, Leningrad, 1954.

[4] I. F. Seits and V. A. Engelgardt, Biokhimiia, 14, No. 6, 487-498 (1949).

[5] V. S. Shapot, Uspekhi Sovremennoi Biol., 34, No. 2, 244-267 (1952).

[6] R. D. Hotchkiss, Advanc. Enzymol., 1944, 4, 153-199.

[7] K. Maruyama, Arch. Biochem. Biophys. 1954, Vol. 52, pp. 485-487.

[8] K. Maruyama, Arch. Biochem. Biophys., 1956, Vol. 60, pp. 74-81.

[9] J. M. Reiner, Arch. Biochem., 1948, Vol. 19, pp. 218-228.

[10] H. E. Robertson and P. D. Boyer, J. Biol. Chem., 1955, Vol. 214, pp. 295-305.

[11] M. L. Tainter, J. Pharm. a. Exptl. Therap., 1934, Vol. 51, pp. 45-58.

THE EFFECT OF PENTAMINE ON THE CARDIOVASCULAR SYSTEM OF DOGS UNDER VARIOUS CONDITIONS OF LONG-TERM EXPERIMENT

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Pharmacologic investigations have shown pentamine* (analogue of the foreign preparation pendiomid) to be one of the most active of modern ganglion-blocking agents. According to I. M. Sharapov's data [1], pentamine exerts marked inhibitory influence on transmission of excitation in autonomic ganglia and lowers their sensitivity to certain pharmacologic substances. At the same time the reactivity of peripheral adrenergic elements is little altered. Pentamine has a definite hypotensive action but enhances pressor reactions to adrenalin. According to the same author, pentamine weakens reflex reactions associated with stimulation of the vagus and sciatic nerves and excitation of the carotid sinus mechanoreceptors. Experiments with cytisine showed that pentamine also diminished the reactivity of the adrenal medulla.

The present work is concerned with the study of the effect of pentamine on the cardiovascular system of dogs under ordinary conditions and against the background of marked arterial blood pressure and ECG changes elicited by conditioned stimuli.

EXPERIMENTAL METHODS

The experiments were performed on 4 dogs, weighing 14-19 kg. Stereotyped motor-defensive conditioned reflexes with electrocutaneous reinforcement were established in all the dogs. The arterial blood pressure was determined in the common carotid artery externalized in a skin flap and records of the ECG were taken; both these determinations were carried out in the conditioned reflex chamber, before and after the experiment. Arterial pressure was determined with the dog standing in the special stand. Three standards leads were used for ECG recording. The reaction to adrenalin was studied in terms of changes in arterial blood pressure and ECG. Adrenalin in 1 : 40,000 dilution was injected into the popliteal vein in the dose of 0.5 ml. Arterial blood pressure and ECG were recorded at the moment of adrenalin injection and 1, 2 and 3 minutes after the injection. Pentamine was given into the popliteal vein, each dog receiving a dose of 1 mg/kg and of 5 mg/kg.

EXPERIMENTAL RESULTS

Pentamine in the doses referred to produce no appreciable changes in the general condition of the dogs or in their behavior. The hypotensive effect in all cases was slight: the systolic and diastolic pressures dropped by an average of 10 mm Hg. In isolated experiments in which the arterial blood pressure exceeded the average normal values the hypotensive effect of 5 mg/kg pentamine was stronger: the pressure fell by 20-30 mm Hg (Fig. 1).

The ECG changes occurring under the influence of pentamine (1 and 5 mg/kg) were in all cases characterized, from the 2nd minute following the injection, by a sharp shortening of the R–R interval (tachycardia) and disappearance of sinus (respiratory) arrhythmia which was observed under ordinary conditions. The P–Q interval was, as a rule, shortened by 0.02-0.05 sec in all the dogs, the voltage of the P wave being increased. In most of the experiments pentamine caused diminution of the positive phase of the T-wave and increase in the T-wave

* Russian trade name.

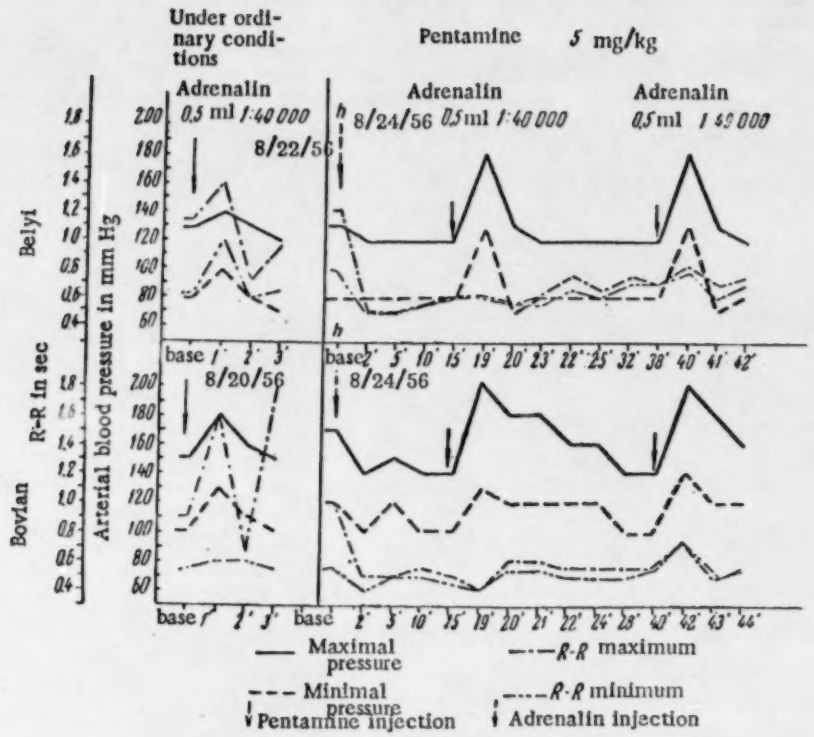

Fig. 1. Effect of pentamine (5 mg/kg) on arterial blood pressure, cardiac rhythm and sensitivity to adrenalin (0.5 ml, 1:40,000).

electronegativity. Injection of adrenalin against the background of pentamine produced more marked rise of blood pressure than under ordinary conditions (Fig. 1). The greatest effect was noted in the dogs Ryzhii and Tarzan which exhibited relatively high sensitivity to adrenalin in the initial period. Thus, while adrenalin, prior to injection of pentamine, produced a rise of blood pressure in Belyi, Bouian, Ryzhii and Tarzan amounting to 10/20, 30/30, 60/30, 40/30 mm Hg respectively, 15-20 minutes after pentamine injection (1 mg/kg) adrenalin produced rises of blood pressure of 30/30, 40/30, 70/60 and 60/50 mm Hg respectively. Repeat test with adrenalin 40-45 minutes after pentamine injection produced less effect, especially with respect to diastolic pressure. Pentamine given in the dose of 5 mg/kg elicited greater increase in sensitivity to adrenalin than that produced by a dose of 1 mg/kg.

Reaction to adrenalin as shown by ECG data following preliminary injection of pentamine (1 and 5 mg/kg) was distinguished from control experiments by the following features.

In all the dogs there was absence of vagus-pulse which usually appears during the 1st minute following injection of adrenalin, and enhancement of sinus arrhythmia. Furthermore, while under ordinary conditions adrenalin caused lengthening of the P–Q interval by 0.03-0.04 seconds (Belyi and Bouian), when pentamine was administered this lengthening was less marked and in some experiments there was even shortening of the P–Q interval by 0.01-0.02 seconds. In the case of the other 2 dogs (Ryzhii and Tarzan) adrenalin caused more pronounced shortening of the P–Q interval (by 0.03-0.04 seconds instead of 0.01-0.02 seconds) when given against the background of pentamine action than in control experiments.

Pentamine also exerted an influence on changes in voltage and direction of the T-wave following injection of adrenalin. When prior to pentamine injection adrenalin produced (in some dogs) diminution and disappearance of the negative phase of the T-wave and increase of the positive, against the background of pentamine this effect was less marked (in Bouian and Ryzhii). If, however, adrenalin produced, before injection of pentamine, diminution of the T-wave electropositivity and increased its negative phase, then following injection of pentamine

the reverse effect was seen: adrenalin, as a rule, increased the T-wave electropositivity.

After studying the effectiveness of the preparation and the distinctive features of its action with regard to the indices of the functional state of the cardiovascular system of dogs under ordinary experimental conditions, we

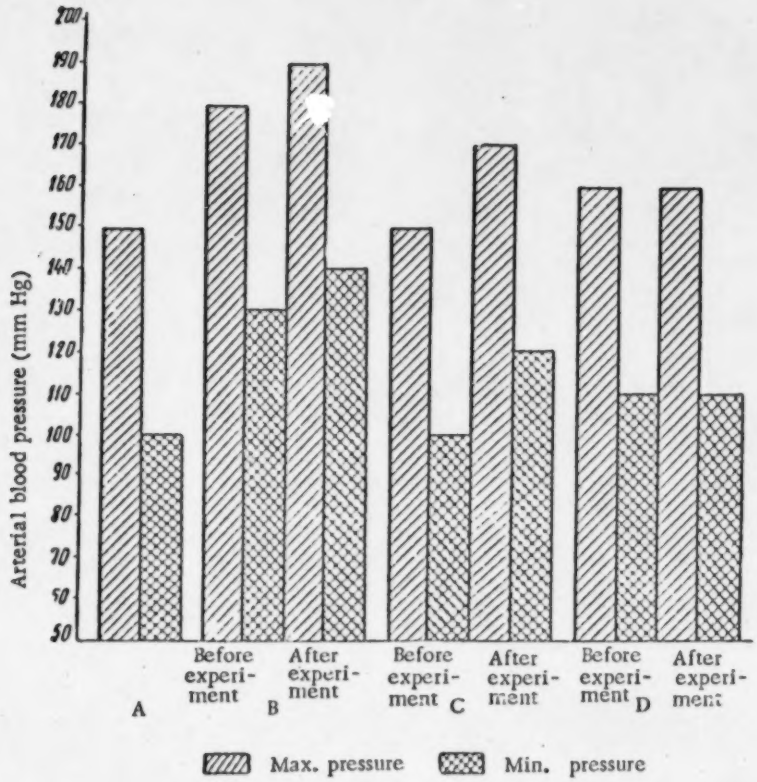

Fig. 2. Effect of pentamine (1 mg/kg and 5 mg/kg) on changes of arterial blood pressure in conditioned reflex chamber. Dog Bouian.
A) Average values of arterial blood pressure outside the chamber; B) changes in arterial blood pressure inside the chamber before and after experiment (without injection of pentamine); C) arterial blood pressures inside chamber after preliminary injection of pentamine in the dose 1 mg/kg; D) the same, dose of pentamine 5 mg/kg.

passed on to the study of the effect of pentamine on the circulatory apparatus against the background of conditioned stimuli. All the dogs had shown marked situational changes in arterial blood pressure and some of the ECG elements (changes in the P–Q and QRST intervals, marked sinus arrhythmia, changes in amplitude and direction of the T-wave) under the influence of functional factors ("confusion" etc.) impinging on them on being placed in the conditioned reflex chamber.

Situational changes in arterial blood pressure were expressed in definite hypertension which appeared immediately upon placing the dogs in the chamber. For example, when arterial blood pressure in the different dogs varied within the limits 120/70-170/120 mm Hg outside the conditioned reflex chamber, it was 150/110-190/140 mm Hg when the dogs were placed in the chamber prior to the experiment. After conditioned stimuli were applied the arterial blood pressure rose still higher, increasing by an average of 20-30 mm Hg.

Situational changes in the ECG consisted of a sharp shortening of the R–R interval (tachycardia), diminution up to complete disappearance of sinus arrhythmia, shortening of the P–Q and QRST intervals and changes in

Effect of Pentamine on ECG Changes in Conditioned Reflex Chamber, Dog Belyi

Date	Experimental conditions	R–R		P–Q	QRS	QRST	S–T	T_2
		Maximum	Minimum					
7/22 1956	Outside conditioned reflex chamber	1.15	0.65	0.16	0.04	0.27	elevated	+0.33
8/4 1956	Inside conditioned reflex chamber (without administration of pentamine) before and	0.55	0.50	0.15	0.03	0.23	elevated	+0.30
	after the experiment	0.55	0.50	0.14	0.03	0.21	"	+0.55
8/26 1956	Inside conditioned reflex chamber 15 minutes after injection of pentamine (5 mg/kg)	0.65	0.60	0.13	0.03	0.24	elevated	+0.37
	before the experiment	0.70	0.65	0.12	0.03	0.23	"	+0.25

Note: Intervals – in seconds; amplitudes of T-wave – in fractions of mv.

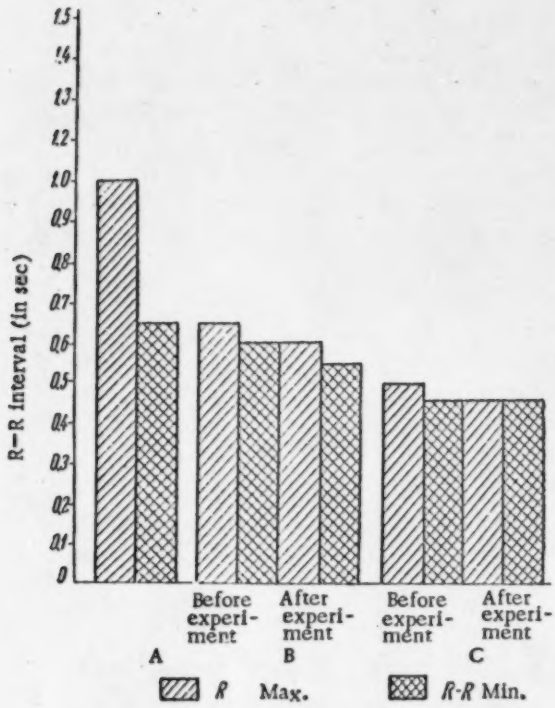

Fig. 3. Effect of pentamine (5 mg/kg) on changes of cardiac rhythm (according to ECG data) in conditioned reflex chamber; dog Bouian.
A) Average duration of the R–R interval outside the chamber; B) change in the R– R interval in the chamber before and after the experiment (without pentamine injection); C) change in R–R intervals in the chamber before and after experiment with preliminary administration of pentamine (5 mg/kg).

amplitude and direction of the T-wave. The use of conditioned signals caused enhancement of the ECG changes observed before the experiment.

The changes in arterial blood pressure and the ECG referred to above (occurring in the conditioned reflex chamber) were altered after preliminary (15-20 minutes before the experiment) injection of pentamine in doses of 1 mg/kg and 5 mg/kg in the following way. In all the dogs pentamine (1 mg/kg) abolished situational hypertension almost completely so that arterial blood pressure values observed in the chamber before the experiment but following preliminary administration of the preparation approached values usually found outside the chamber (Fig. 2). The use of conditioned stimuli, however, produced the same effect as in the control experiments, viz. a rise of blood pressure by 10-20 mm Hg; in other words, pentamine in the dose 1 mg/kg did not abolish completely the pressor influences associated with the use of conditioned stimuli. Pentamine in the dose 5 mg/kg abolished not only the situational hypertension but also, in the majority of cases, exerted a clear inhibitory influence on the development of the pressor effect associated with the use of conditioned stimuli.

Pentamine did not suppress the situational ECG changes which occurred inside the chamber. This is understandable, since it was shown earlier that the preparation itself produced in all the animals marked tachycardia, disappearance of sinus arrhythmia and shortening of the P–Q and QRST intervals.

The data obtained thus indicate that pentamine possesses slight hypotensive action and marked effect with respect to the ECG, causing tachycardia, disappearance of sinus (respiratory) arrhythmia, shortening of the P–Q and QRST intervals and alteration in the amplitude and direction of the T-wave. Pentamine potentiates the pressor effect of adrenalin and suppresses the vagus-pulse usually observed under the action of adrenalin. Pentamine in the dose of 1 mg/kg abolishes situational hypertension (in conditioned reflex chamber) and in the dose of 5 mg/kg also inhibits pressor effects connected with the use of conditioned stimuli.

SUMMARY

It was established in experiments on 4 dogs that pentamine, introduced intravenously in doses of 1 mg per kg of body weight and 5 mg per kg has a slight hypotensive effect. On the other hand it has a pronounced influence on ECG, causing tachycardia, disappearance of sinus (respiratory) arrhythmia, shortening of P–Q and QRST waves, as well as the change of the amplitude and direction of T-wave. Pentamine increased the pressor effect of adrenalin and depressed the vagus pulse which was usually noted upon administration of adrenalin. Pentamine in the dose of 1 mg per kg removes situational hypertension (in conditioned reflex chamber). The dose of 5 mg per kg, besides giving the effect referred to, inhibits the pressor action connected with the use of conditioned stimuli.

LITERATURE CITED

[1] I. M. Sharapov, Farmakol. i Toksikol., 18, No. 1, 8-13 (1955).

[2] H. I. Bein and R. Meier, Schweiz. med. Wschr., 1951, No. 19, pp. 446-450.

THE SELECTIVE EFFECT OF SOME CHEMICAL STIMULI ON REFLEXES FROM CHEMORECEPTORS

COMMUNICATION VI. THE EFFECT OF STREPTOMYCIN ON REFLEXES FROM SPLEEN, KIDNEY AND HIND LIMB CHEMORECEPTORS

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In the earlier publications from this laboratory [1, 2, 3, 4] it was shown that streptomycin, given parenterally, had the ability to cause profound depression of reflexes from the small intestine and carotid sinus chemoreceptors. On the other hand, reflexes arising upon stimulation of the urinary bladder mechanoreceptors and those of the central portion of the tibial nerve proved to be very resistant to the inhibitory effect of the antibiotic.

The present communication presents data of further studies on the effect of streptomycin on interoceptive reflexes from the spleen, kidney and the hind limb.

EXPERIMENTAL METHODS

Experiments were performed on cats under urethane anesthesia. Stimulation of the spleen, kidney and hind limb chemoreceptors was achieved in the following way. The organ was subjected to vascular isolation by the V. N. Chernigovskii technique; the organ then derived its nutrition from oxygenated Tyrode solution. The isolated organ preserved its connection with the organism only by means of its nerves. Acetylcholine or carbon dioxide was then added to the perfusion fluid, thus achieving stimulation of the chemoreceptors.

In some of the cases the tibial nerve was simultaneously exposed in the lower third of the shank (in experiments with perfusion of the lower limb the nerve was exposed on the side of vascular isolation) which was then subjected to induction current stimulation.

Streptomycin (the sulfate or calcium chloride complex) was injected intramuscularly or intravenously after the initial background of chemoreceptor excitability had been established. At different intervals after injection of the antibiotic the reflexes from chemoreceptors were again reproduced, and in a number of cases this applied also to reflexes arising from stimulation of the tibial nerve.

EXPERIMENTAL RESULTS

Effect of streptomycin on reflexes from spleen chemoreceptors. In 7 control experiments it was established that the excitability of chemoreceptors in perfused spleen with respect to the stimuli used was sufficiently high and that it was maintained without appreciable changes for as long as $1\frac{1}{2}$-3 hours during which observations were made.

The effect of streptomycin on reflexes from the spleen chemoreceptors was studied in 17 experiments. The antibiotic was given intramuscularly: in 3 experiments in doses of 250,000 units, in 9 experiments in doses of 350,000 units and in two experiments in doses of 500,000 units. In 3 experiments streptomycin was injected into the femoral vein in doses of 36,000, 60,000 and 100,000 units.

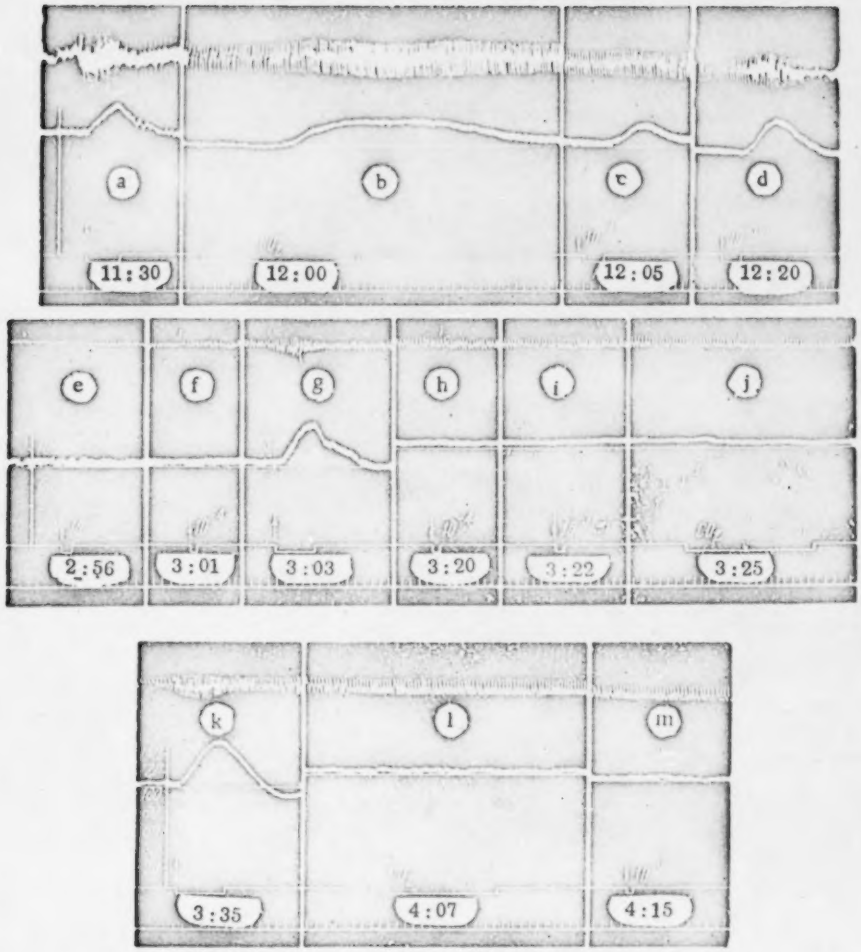

Fig. 1. Effect of streptomycin on reflexes from chemoreceptors in perfused spleen and tibial nerve.
a), b), c), d), e) Initial reflexes from the tibial nerve and spleen chemoreceptors (to various stimuli); f), g), h), j), k), l), m), o), p) reflexes from the spleen chemoreceptors after administration of streptomycin (injected at 12:25 intramuscularly in the dose of 350,000 units and at 2:45 intravenously in the dose of 100,000 units); i), n) reflex from the tibial nerve after administration of streptomycin (increased by 48 and 63%). Records from above down; respiration, blood pressure, base line, stimulus mark, time marker (5 seconds); ↑ – stimulation of the tibial nerve; ↓ – acetylcholine injection. Experiment 189, May 20, 1954. Cat weighing 3.2 kg.

In all the experiments the magnitude of the reflex from spleen chemoreceptors to acetylcholine in dilution 1×10^{-4} (1 ml) was diminished under the influence of streptomycin. In most cases (9 out of 13) the reflex to acetylcholine proved to be either completely inhibited or inverted, i.e., depressor instead of pressor; the reflex was reduced by 87% in one case, by 33-44% in three cases.

The excitability of the spleen chemoreceptors with respect to acetylcholine (1×10^{-4}) proved to be reduced in most experiments as soon as 10-40 minutes after administration of the antibiotic; this was observed in 11 out of 13 cases. Maximal inhibition of the reflex to acetylcholine in the majority of experiments (9 out

of 13) occurred within the first hour following intramuscular injection of the antibiotic. Intravenous injection of the antibiotic inhibited the reflexes more rapidly. In these cases the described effect appeared when minimal doses of streptomycin (60,000-100,000 units per cat) were used.

The effect of streptomycin on reflexes elicited by acetylcholine in 1×10^{-3} dilution (1 ml) was studied in 15 experiments. The reflex to acetylcholine was reduced in most cases (13 out of 15) under the influence of streptomycin. In 6 experiments it disappeared completely, in 2 it was reduced by 75-76%, in another 2 by 50-58% and in 3 by 40-45%. Maximal inhibition of the reflex to acetylcholine (1×10^{-3}) following intramuscular administration of the antibiotic occurred in most cases within the first or second hour, and in the course of 2-2½ hours in the remainder.

In 15 other experiments a study was made of the effect of streptomycin on reflexes from the spleen chemoreceptors to carbon dioxide. In 9 cases the reflex disappeared completely or underwent inversion on injection of the antibiotic, decreased considerably in 4 (by 52-75%), decreased by only 13% in one and was unchanged in one case only. Maximal inhibition of the reflex to carbon dioxide following intramuscular injection of the antibiotic was observed during the first or second hour in 9 experiments. Inhibition of the reflex was more rapid when the preparation was injected intravenously.

Finally, in 7 experiments of this series a parallel study was made of the excitability of the spleen chemoreceptors to one or another stimulus and of reflexes elicited by electric current stimulation of the tibial nerve. In 4 experiments the reflex from the tibial nerve was decreased, but only slightly, under the influence of streptomycin. Maximal reduction of the reflex was by 6.14 and 20%, and only in a single case did it reach 60%. In 3 experiments the reflex was, on the contrary, increased (by 19, 42 and 63%).

Parenterally administered streptomycin thus exerts a marked inhibitory effect on reflexes from the chemoreceptors of perfused spleen connected with the organism by nerves only. Unlike the spleen chemoreceptor reflexes, those from the tibial nerve either increased or decreased, although usually to a considerably lesser extent, under the influence of streptomycin (Fig. 1). It follows from these data that the tibial nerve reflex is more resistant to the action of the antibiotic than the reflexes from the spleen chemoreceptors. Detailed analysis of the data obtained revealed that reflexes from chemoreceptors were inhibited to a much greater degree than reflexes from the nerve; this held true for those cases in which the initial magnitude of these reflexes was the same.

Stimulation of the spleen chemoreceptors led, in a number of cases, to changes in respiration. Streptomycin as a rule inhibited not only the vasomotor but also the respiratory component of the reflex from these chemoreceptors.

The effect of streptomycin on reflexes from kidney chemoreceptors. This effect was studied in 9 experiments. The antibiotic was given intramuscularly in 2 cases in doses of 300,000 -400,000 units, intravenously in seven in doses of 70,000-200,000 units.

In 2 experiments nicotine in 1×10^{-6} dilution served as stimulus for renal chemoreceptors. Under the influence of streptomycin the reflex diminished by 75% in one case and was completely inhibited in the second.

In 4 experiments nicotine in 1×10^{-5} dilution was used as stimulus for renal chemoreceptors. Under the influence of streptomycin the reflex to nicotine diminished by 50% in one case, by 80% in another and in the remainder it was completely suppressed.

The effect of streptomycin on reflexes from renal chemoreceptors to nicotine in 1×10^{-4} dilution was studied in 2 experiments. In one case the reflex was reduced by 88%, in the other it disappeared completely.

The effect of streptomycin on reflexes elicited by 1×10^{-3} dilution of nicotine was studied in 3 experiments. In 2 cases the reflexes disappeared completely under the influence of the antibiotic and in one case there was 92% reduction.

Maximal inhibition of reflexes from renal chemoreceptors to the stimuli mentioned above appeared within 40 minutes – 1½ hours when streptomycin was given intramuscularly and after 20-40 minutes when it was given intravenously.

In 2 experiments the threshold of the vascular reflex to stimulation of the tibial nerve was determined during the period of inhibition of reflexes from the renal chemoreceptors; the threshold proved to be equal to 16 cm in

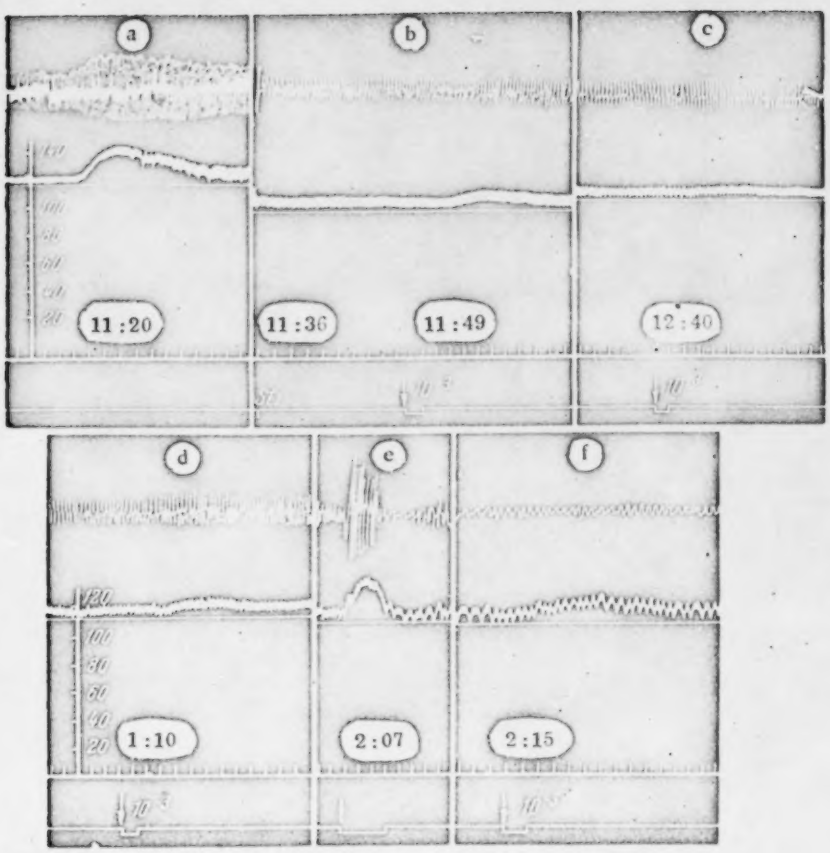

Fig. 2. Effect of streptomycin on reflexes from perfused kidney.
a) Initial reflex; b), c), d), f) after administration of streptomycin (intravenous injection at 11 : 36 in the dose of 100,000 units); g) reflex to stimulation of tibial nerve. Records from above down: respiration, blood pressure, base line, time marker (5 seconds), stimulus mark. ↑ – stimulation of tibial nerve; ↓ – acetylcholine injection. Experiment 419, September 2, 1955. Cat weighing 2.8 kg.

one experiment and 15 cm in another (in control experiments the threshold was 14 and 17 cm respectively). These data indicate preservation of high excitability of the vasomotor center against the background of depression of reflexes from renal chemoreceptors (Fig. 2).

The respiratory reflex arising from stimulation of chemoreceptors was depressed to the same extent as the vasomotor reflex under the influence of streptomycin.

Effect of streptomycin on reflexes from tissue chemoreceptors of the hind limb. It was established in 5 preliminary control experiments that the magnitude and character of reflexes from tissue chemoreceptors of the limb remained substantially unaltered throughout 2-4 hours of observation when they were subjected to repeated action of the stimuli used (acetylcholine and tuberculin); the same was true for reflexes from the tibial nerve.

The effect of streptomycin on reflexes from the chemoreceptors of the limb elicited by acetylcholine in 1×10^{-3} and 1×10^{-4} dilution was studied in 13 experiments. In all experiments without exception the effect of the antibiotic was to depress the reflexes to acetylcholine (by an average of 79.5%). In 4 experiments the reflex to acetylcholine disappeared entirely or was inverted; in 5, depression of reflexes by more than 75% was observed, in two, by 50% and over, in two others by 40 and 36%. Depression of reflexes to acetylcholine became

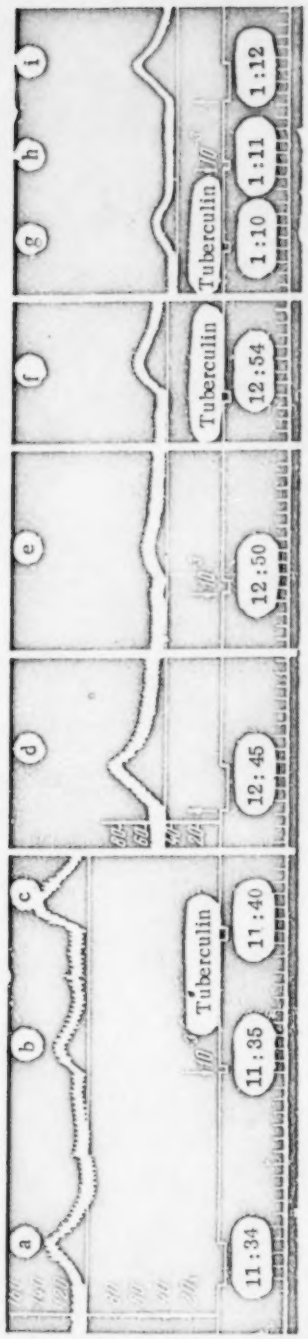

Fig. 3. Effect of streptomycin on reflexes from chemoreceptors and tibial nerve of perfused limb.
a), b), c) initial reflexes; e), f), g), h) reflexes from chemoreceptors after administration of streptomycin (intramuscular injection at 11:43 in the dose of 500,000 units); d), i) tibial nerve reflexes. Records from above down: blood pressure, base line, stimulus mark, time marker (5 seconds). Experiment 116, January 5, 1954. Cat weighing 3.6 kg.

maximally apparent during the first hour in 6 cases and during the second hour following administration of the antibiotic in the remainder.

In all the 13 experiments the effect of streptomycin on the magnitude of reflexes from the tibial nerve of the perfused limb was also studied. In 4 experiments the reflex from the nerve diminished by 20-30%, in 3 by 36-40%, in 5 by 41-76%; in one experiment the reflex increased. Maximal depression of the reflex from the tibial nerve was noted after 20-60 minutes in 4 experiments, in the remainder after 1-2 hours following administration of the antibiotic. It must, however, be pointed out that the reflex from the tibial nerve was not depressed by streptomycin consistently and far from every time the stimulus was applied. The reflex referred to was diminished in 29 out of 44 tests. In seven of these observations the diminution of the reflex was extremely slight (less than 15%). Unlike this, the reflex from the limb chemoreceptors to acetylcholine after administration of streptomycin proved to be diminished in the overwhelming majority of cases (46 out of 49), slight diminution (less than 15%) being noted in only 7 of 46 cases.

It must also be pointed out that in 7 out of 13 experiments with perfusion of the limb, no changes in the reflexes from the tibial nerve were noted during the first phase of the antibiotic's action while in the remainder the reflex even showed an increase of 5-39%, a phenomenon not observed in the study of reflexes elicited by acetylcholine.

Finally, comparison of the effect of streptomycin on reflexes from the tibial nerve and to stimulation of chemoreceptors by acetylcholine applied to each experiment showed that reflexes from chemoreceptors were depressed much more rapidly and to an immensely larger extent (Fig. 3). Analysis of the whole material revealed that in the main only during the late stages of streptomycin's action, especially when large doses of the antibiotic were used, the profound depression of reflexes to acetylcholine was associated with depression of reflexes from the tibial nerve as well, which did not, however, in a single experiment reach the same level as that observed for chemoreceptors.

The data obtained thus showed that parenteral administration of streptomycin in most cases caused marked weakening or complete depression of reflexes from the chemoreceptors of the spleen, kidney and hind limb. This effect occurred more rapidly when the antibiotic was given intravenously than intramuscularly.

The depression of reflexes from chemoreceptors was persistent and remained for $1\frac{1}{2}$-2 hours when streptomycin was given intramuscularly; the reflexes began to recover gradually after this period of time. The effect was rather more short-lived when the antibiotic was injected intravenously.

Streptomycin depressed both the vasomotor and the respiratory reflexes arising from stimulation of chemoreceptors.

Unlike the chemoreceptor reflexes, reflexes arising from stimulation of a somatic afferent nerve (tibial) tended to increase or remained unchanged under the influence of streptomycin. In some cases these reflexes were diminished. The latter was observed during later periods and the depression of these reflexes was less marked, being relatively transient and appearing when large doses of streptomycin were used. These data indicated that the excitability of the vasomotor and respiratory centers to afferent impulses from a peripheral somatic nerve did not, in most cases, decrease significantly under the influence of the antibiotic. It must also be stressed that under the experimental conditions employed the antibiotic did not come into direct contact with the tissue chemoreceptors of the spleen, kidneys and the limb from which reflexes to chemical stimuli were elicited. Consequently, both the receptors themselves and the efferent part of the reflex arc were not subjected to the action of streptomycin. It follows from this that the depressing effect of the antibiotic on reflexes from chemoreceptors is associated with its blocking influence on that portion of the afferent part of the reflex arc which is situated within the central nervous system.

SUMMARY

Short-term experiments on cats under urethane anesthesia were performed. The effect of streptomycin introduced parenterally was studied on reflexes from chemoreceptors of the spleen, kidney and hind limb. Chemoreceptors were stimulated by acetylcholine, nicotine and carbon dioxide which were introduced into the fluid perfusing these organs, isolated from the general circulation but connected to the organism by the nerves. It was established that streptomycin in a dose of 60,000 to 200,000 units completely eliminates or markedly depresses the reflexes from chemoreceptors when introduced intramuscularly; the maximal effect of the antibiotic is most frequently seen at the end of the first hour or during the second following the intramuscular injection, and during the first hour after intravenous injection. However, the reflexes which appeared in stimulation of the central portion of the large tibial nerve by induction current in the same experimental conditions were different from the reflexes coming from chemoreceptors. They either did not change at all, or showed only slight increase or decrease. Sometimes these reflexes were subjected to pronounced depression. However, this occurred only when large doses of the antibiotic were used at the late stages of its action and to a lesser degree than the reflexes from the chemoreceptors. The authors comes to the conclusion that streptomycin has a marked neurotropic effect, which is manifested in depression of the part of the chemoreceptor reflex arc located in the central nervous system.

LITERATURE CITED

[1] I. E. Gaber and G. S. Kan, Biull. Eksptl. Biol. i Med., No. 9, 48-53 (1953).

[2] I. E. Gaber, In the symposium: The Role of the Nervous System in the Pathogenesis and Treatment of Tuberculosis,* 1954, pp. 320-329.

[3] I. E. Gaber, Biull. Eksptl. Biol. i Med., 40, 25-28 (1955).

[4] G. S. Kan, Texts of Communications Presented at the Second All-Union Conference of Pathophysiologists,* Kiev, 1956, pp. 154-156.

* In Russian.

CIRCULATORY AND RESPIRATORY CHANGES ASSOCIATED WITH THE ACTION OF SOME CHEMICAL SUBSTANCES ON CEREBRAL MENINGES

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Many observations have been accumulated in the practice of neurosurgery which attest to the possibility of serious impairment of circulation and respiration associated with trauma of the cerebral meninges. Reflex changes of blood pressure and respiration have been described in a number of research papers dealing with experimental mechanical stimulation of the cerebral meninges [2, 3, 6, 7, 9].

Investigation of the sensitivity of the meninges to chemical stimuli is of particular interest since many medicinal substances possessing certain chemical structures are introduced into the CSF and therefore come in contact with the cerebral meninges. It is also known that the relatively constant chemical composition of the cerebrospinal fluid is ensured by special physiologic adaptations which prevent the passage of a number of substances into the CSF. However, under certain conditions such as pathologic states, changes in the permeability of biologic barriers may lead to disturbances of the chemical composition and biologic properties of the cerebrospinal fluid. It may be supposed that sensitive elements are present in the meninges which react to changes in the composition of the cerebral milieu interieur, viz. the cerebrospinal fluid [4].

EXPERIMENTAL METHODS

Experiments were performed on 50 dogs under short-term and long-term conditions. Free access to the dura was achieved by operative means. Trephining was carried out under morphine-ether anesthesia. Part of the cranial bone was removed in such a way as to leave the dura intact. The edges of the bone defect were smoothed down with nippers, continuous catgut sutures were placed in the soft tissues and a colloid dressing applied to the defect. A "window" was thus made in the skull to be used for observations under long-term conditions. In short-term experiments the same operation was carried out under urethane anesthesia but the trephining was more extensive. Stimulation of the cerebral meninges was performed under a depth of anesthesia at which the corneal reflexes were preserved.

The following substances were used as chemical stimuli: acetylcholine and nicotine in dilutions of 1 : :10,000, adrenalin 1 : 1,000, 4% solution of pyramidon, 40% solution of glucose, lobeline 1 : 250. All these substances were introduced in small amounts (0.2-0.5 ml) under the dura at various sites. Some of the observations were made after preliminary introduction of 0.5% solution of novocain or cocaine into the subdural space. The other group of control experiments was made up of observations with subdural administration of 0.9% sodium chloride solution.

Reflex reactions were assessed by recording the electrocardiogram, blood pressure (mercury manometer), rate of blood flow (lobeline test) and respiration.

EXPERIMENTAL RESULTS

The results obtained may be summarized briefly as follows: the effect of adrenalin (36 stimulations) was expressed in acceleration of the blood flow, a pressor-depressor reaction of the blood pressure and changes in the electrocardiogram indicating increased contractile power of the heart muscle (Fig. 1).

Fig. 1. Changes in respiration (top trace) and blood pressure (middle trace) following subdural introduction of 0.3 ml 1 :1,000 solution of adrenalin in the fronto-parietal area (moment of injection denoted by arrow).
The two bottom traces are blood pressure base line and time marker (1 second). Short-term experiment, December 22, 1955. Female dog No. 24 weighing 9.7 kg.

The action of pyramidon on the meninges (36 stimulations) led to slowing of the blood flow and some fall in blood pressure; the electrocardiogram indicated impairment of coronary circulation and weakening of cardiac contractions (Fig. 2 and 2,a).

Stimulation of the cerebral meninges by solutions of acetylcholine and nicotine under short-term experimental conditions (43 stimulations) was accompanied by a rise of blood pressure. As a rule, parallel to the rise of blood pressure, changes also occurred in respiration shown on the kymogram as increased respiratory rate and increased amplitude. The electrocardiographic changes were characterized by tachycardia, shortening of the S-T interval and increased T-wave. In isolated experiments with subdural introduction of nicotine, paroxysmal tachycardia was noted (Fig. 3 and 3,a). No characteristic changes in the rate of blood flow were seen in this series of experiments.

Subdural introduction of glucose (42 trials) led to no substantial changes of circulation or respiration either in short-term or prolonged experiments. The recorded indices remained unchanged when larger amounts of glucose were given subdurally.

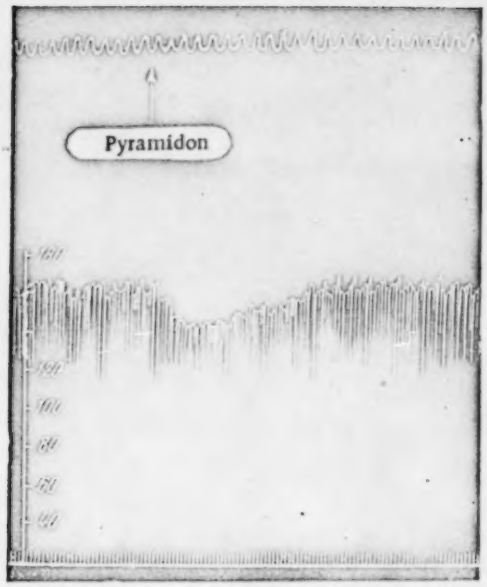
In the case of some of the chemical substances tested (pyramidon, lobeline) increasing the amount of solution given subdurally led to the development of convulsive paroxysms with an acute onset and relatively rapid termination. This phenomenon was noted only in the prolonged experiments in the case of lobeline.

The experiments thus show that more or less pronounced changes in circulation and respiration occur in response to chemical stimulation of the cerebral meninges. The intensity of the effects produced by similar stimuli in different experiments was different, but the character and direction of the ensuing changes remained the same.

We are inclined to the view, based on our observations, that the changes occurring upon chemical stimulation of the cerebral meninges are reflex and arise as the result of stimulation of the meningeal receptor apparatus. This view is supported by the fact that novocain or cocaine anesthesia of peripheral receptor elements followed by the action of chemical stimuli was accompanied by extremely slight reactions or no changes at all. The rapid onset and short duration of the changes also favor their reflex character. Finally, this view is also confirmed by experiments in which acetylcholine and nicotine stimulation was followed by a rise of blood pressure. It is well known that resorptive action of these substances leads to lowering of blood pressure.

It was noticed in the course of the experiments that application of the various chemical substances to the surface of the dura and the pia did not produce the described effects while sub-meningeal introduction of the same substances led to definite changes. This demonstrates that the receptors are situated predominantly on inferior surface of the dura.

In order to effect stimulation of the inner surface of the dura a dural flap was turned and the chemical substances being tested were applied to its inner surface. This led to effects similar to those obtained on subdural introduction of the substances but less marked. This considerable diminution in the intensity of response reaction is, in our opinion, connected with interruption of nervous conductors during the turning of the flap. The possibility is not excluded that the more pronounced effects observed on subdural administration of the chemical substances is determined by the complex action exerted when this method of introduction is used (large area, increase in pressure, absence of lowering of sensitivity produced by preliminary deprivation of the dura of its usual environment etc).



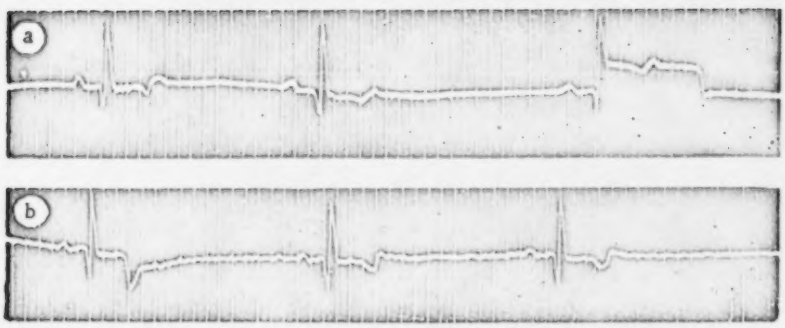


Fig. 2. Changes in respiration (top trace) and blood pressure (middle trace) following subdural introduction of 0.5 ml 4% solution of pyramidon in the occipital area (moment of injection denoted by arrow).
Bottom trace – time marker (1 second). Short-term experiment, December 12, 1955. Male dog, No. 4 weighing 17.5 kg. Electrocardiograms: a) before pyramidon injection; b) after injection. Lead II.

The meningeal sensory elements which can be considered as mechanoreceptors on morphological grounds can, under certain conditions, respond to chemical stimuli also. The fact that the observed changes which follow the action of chemical substances on the cerebral meninges are connected with stimulation of nervous elements situated predominantly on the inner surface of the dura is in accord with the modern concepts [1, 5] which stress the barrier role of the pia and selective permeability of chemical substances from the subdural space into the cerebrospinal fluid. It is quite possible that the described properties of the meningeal receptor elements assist the reflex regulation of the chemical composition of the cerebrospinal fluid. The view that reactions to chemical stimuli might result from sensitivity of nonspecific chemoreceptors was expressed before us by V. N. Chernigovskii [3] with respect to sensory nervous elements of other receptor zones.

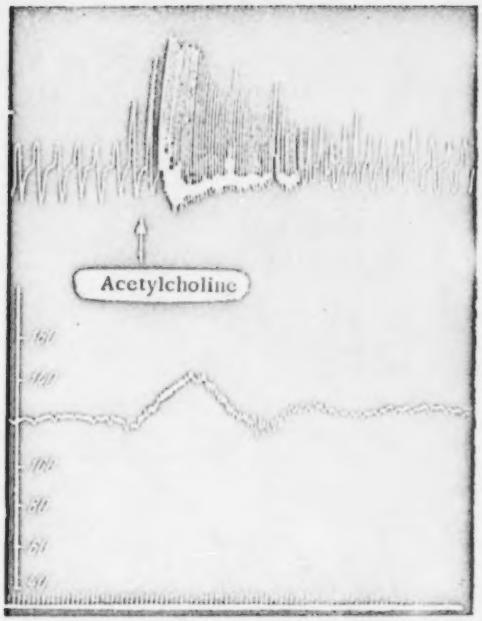


Fig. 3. Changes in respiration (top tracing) and blood pressure (middle tracing) following subdural introduction of 0.5 ml 1 : 10,000 solution of acetylcholine in the temporal area (moment of injection denoted by arrow).
Bottom tracing – time marker (1 second). Short-term experiment, July 27, 1955. Male dog, No. 9 weighing 18.5 kg.

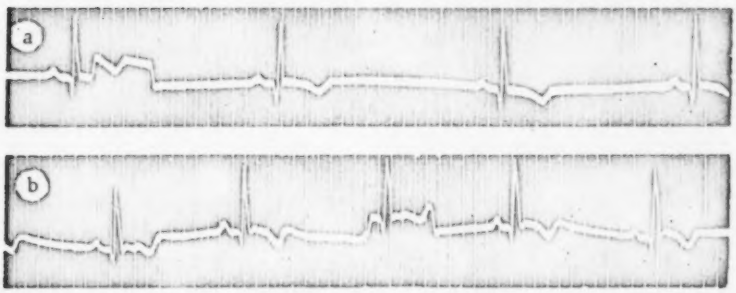


Fig. 3a. Electrocardiograms (Lead II); experiment on July 27, 1955.
a) Before acetylcholine stimulation; b) after stimulation.

SUMMARY

Excitation of brain meninges by chemical substances was carried out in 50 dogs in short-term and prolonged experiments. The following solutions were used: acetylcholine and nicotine – 1 : 10,000, lobeline – 1 : 250 adrenalin – 1 : 1,000, 4% pyramidon solution and 40% solution of glucose. These solutions were introduced into various areas of the dura mater in the dose of 0.2-0.5 cc. Electrocardiogram tracings were taken, blood pressure, the velocity of blood flow and respiration were recorded. Adrenalin caused acceleration of the blood flow, pressor-depressor reactions of the blood pressure and changes in the electrocardiogram which denoted increased contractile

power of the heart muscle. Pyramidon diminished the velocity of blood flow and reduced the blood pressure. Electrocardiogram showed disturbance of the coronary circulation. Acetylcholine and nicotine caused increase of respiration frequency and an increased amplitude of the respiratory movements which were the result of the rise in blood pressure. The above reactions are reflexes, as they disappear following the preliminary subdural introduction of novocain.

LITERATURE CITED

[1] I. A. Alov, "Circulation of Fluid in the Subdural Space of the Brain and the Barrier Function of its Arachnoid,"* Thesis. Moscow, 1947.

[2] D. A. Biriukov, In the book: Material Concerning Reflex Regulation of the Cardiovascular System," Voronezh, 1946.

[3] D. A. Biriukov, Fiziol. Zhur. USSR, 34, No. 6, 689-694 (1948).

[4] T. V. Vetrenko, Vrachebenoe Delo, No. 3 (1956).

[5] M. S. Stanislavskaia, "Permeability of Penicillin Through the Serous, Sinovial and Spinal Cord Membranes and the Effect of Massive Doses of Penicillin on Hemopoiesis."* Thesis, 1950.

[6] A. M. Ugolev and V. M. Khaiutin, Collected Works of Students of the III Faculty of the Naval Medical Academy." Leningrad, 1948, Vol. 4, pp. 30-39.

[7] M. I. Kholodenko, Fiziol. Zhur. SSSR, 38, No. 1, 46-52 (1952).

[8] V. N. Chernigovskii, "Investigation of Visceral Receptors,"* Thesis, Leningrad, 1941.

[9] Maassland and Saltikoff, cited by: T. Kocher, Nothnagels Handbuch specielle Pathologie und Therapie, Wien, 1901, Vol. IX, pp. 113-134.

*, In Russian.

MICROBIOLOGY AND IMMUNITY

THE PROBLEM OF DETECTION OF TISSUE ANTIGENS

COMMUNICATION II. ON THE CONDITIONS OF DETECTION OF COMPONENTS OF ANTIGENIC MIXTURES BY ANAPHYLAXIS

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When using the anaphylactic reaction to distinguish tumor antigens from the antigens of normal tissues, it is necessary after sensitizing the animal to a tumor preparation to desensitize it to normal antigens, and only after this, proceed with the test injection of the tumor preparation. Therefore two questions arise: 1) how to carry out the desensitization to be confident of its completeness; 2) how does such desensitization with respect to the predominant antigen affect the subsequent reaction of the animal to a "minor" antigen, i.e., one making up only an insignificant portion of the antigenic complex?

To answer the first question it is necessary to ascertain the relation between the shocking and desensitizing dose.

METHODS

For this purpose guinea pigs were sensitized to horse serum, which we injected in the amount of 0.5 ml per 100 g body weight (or 120 mg protein for a 300 g animal). After 3 weeks we titered out the minimum dose of antigen which when injected (intravenously) would produce fatal anaphylactic shock. This proved to be 0.01 ml of serum per 100 g body weight. Proceeding from this, experiments were carried out to investigate the conditions of desensitization, the results of which are presented in the table.

RESULTS OF EXPERIMENTS

From the table it may be seen that following the (intravenous) injection of antigen in the amount of 3-4 times the fatal dose, three out of 19 guinea pigs succumbed; the majority of the remainder responded with a pronounced anaphylactic reaction after which they showed themselves desensitized to 3 and even to 5 fatal doses. However, animals into which we injected 10 shocking doses to test the completeness of desensitization, gave a definite reaction, and pigs receiving 30 and more doses succumbed.

Only after double desensitization (not less than 5 shocking doses) did the insensitivity reach 30-35 doses, which contained 60-70% of the amount of antigen used for the sensitization.

It must be mentioned that about a day after double desensitization the sensitivity of the pigs was noticeably restored, and they reacted to 5 shocking doses.

In another analogous experiment the sensitization was carried with a so-called "weak" antigen, i.e., a preparation of nucleoprotein from horse liver in the amount of 1 ml, i.e., 20 mg protein for a pig weighing 300 g. In this case the shocking fatal dose was relatively large – from a half to all the sensitizing dose.

On desensitizing with half of the lethal dose a definite anaphylactic reaction was observed; such a reaction was observed in 5 of 8 guinea pigs into which we injected 1/5 of the shocking dose. All of these animals after

Relation Between Shocking and Desensitizing Doses

No. of pig	Reaction to desensitization with 3-4 MLD	Investigation of completeness of desensitization by various dosages			Repeated attempt at desensitization 30 MLD	Reaction after 24 hours to 5 MLD
		3-5 MLD	10 MLD	30 MLD and more		
1	Died					
2	"					
3	"					
4	+++			Died		
5	+++					
6	+++					
7	+++		++		−	++
8	+++	±			−	++
9	++		++		−	++
10	++	−			−	++
11	++	−			−	++
12	++	+			−	
13	++	−			++	
14	++	−			+	
15	++	+			+ (++)	
16	+ (++)	±			+	
17	+ (++)	±			+ (++)	
18	+ (++)	+			+	
19	+	+			+ (++)	

Explanation: +++ pronounced anaphylactic shock with severe respiratory difficulties, loss of ability to stand, convulsions; ++ definite anaphylactic reaction with pronounced symptoms of bronchial spasm (convulsive coughing); + anaphylactic symptoms in the form of scratching, sneezing, running motions, without definite symptoms of bronchial spasm; ± negligible reaction; − no symptoms.

this showed themselves to be desensitized to a repeated injection of half of the shocking dose, but on the third injection of antigen in the amount of 2-4 lethal doses 3 pigs out of 9 reacted. In addition it was also noticed in this experiment that, even after triple desensitization, among a part of the guinea pigs after 24 hours sensitivity to the antigen was restored, and they reacted to a single shocking dose.

For investigation of the second question – the effect of complete desensitization to the predominant major components of the antigen complex on the sensitivity of the animals to minor components – guinea pigs were sensitized with a mixture of human serum and horse serum (two "strong" antigens), mixed in the proportions of 50 : 1 (0.5 ml human serum to 0.01 ml horse serum per 100 g body weight). The shocking dose to the "minor" antigen, i.e., horse serum, amounted, for animals not subjected to desensitization, to 0.01 ml per 100 g of body weight. After profound desensitization to the "major" antigen (in this case, to horse serum) the animals did not react to this dose of antigen or even to three times this amount, and only with doses increased 6-10 times (0.06-0.1 ml per 100 g of animal) was it possible to record a definite reaction.

Another series of experiments gave analogous results; in these the animals were sensitized with a mixture of horse and rat serum, also in the proportions of 50 : 1, but in this case the "minor" antigen was rat serum, the "major," horse serum. The shocking dose of the "minor" antigen in this case amounted to 0.025 ml per 100 g of body weight, and for the "major" antigen amounted to 0.01 ml. After triple desensitization of the animals to the "major" antigen with increasing doses, beginning with 3/4 of the shocking dose of the antigen in question, loss of sensitivity to the horse serum was achieved. During this the sensitivity to the "minor" antigen also decreased, so that a definite anaphylactic reaction could be obtained only by the injection of 0.1 ml and more of rat serum.

In a third series of experiments guinea pigs were sensitized to two "weak" antigens, i.e., to nucleoprotein from organs of horse and rat; these were mixed in the proportions of 50:1 (horse nucleoprotein in the amount of 20 mg of protein and rat in the amount of 0.04 mg of protein, for an animal of 300-350 g weight). The shocking (lethal) dose to the "major" antigen in this case reached 1 ml of the preparation (20 mg of protein).

On the first desensitization with 0.25 ml the guinea pigs gave a definite anaphylactic reaction (++); even on the injection of 0.1 ml such reactions were observed in 5 out of 8 animals. Repeated injection of 0.25 ml did not produce a reaction; no reaction was observed either in pigs into which in a third of the experiments we injected 1 ml of the preparation, but at a dose of 2 ml, 2 pigs out of five reacted (+ and ++). In respect to sensitivity to the "minor" antigen, those pigs which were not subjected to desensitization to the "major" antigen died on the injection of 1-2 ml of rat nucleoprotein. A dose of 1.5-2 ml elicited severe shock and death of the animals within a few minutes, but on the injection of 1 ml of the preparation the animals responded by slow shock; death occurred in the course of 8-10 hours. Smaller doses (0.5 and 0.25 ml) elicited a definite anaphylactic reaction (++) which led to the death of the animal.

For guinea pigs subjected to triple desensitization to horse nucleoprotein, the lethal dose of rat antigen did not increase, and they succumbed following the injection of 1-2 ml of the corresponding preparation. But not all animals reacted to smaller doses (0.5 and 0.25 ml).

DISCUSSION

From the foregoing material it follows that sensitizing animals to a complex antigen and employing desensitization from removal of the sensitivity to one component, it is necessary, in the first place, to carry out desensitization starting with a shocking dose of the component in question, and, in the second place, establish the completeness of desensitization to not less than 10 shocking doses (preferably to values larger than this). In such work one must consider that repeated desensitization to the antigen which appears to predominate quantitatively leads to a decrease in the sensitivity of the animals to the "minor" antigen, which forces an increase in the dose of the preparation used in the test injection. Consequently the situation with tumor preparations, referred to above, is more complicated, since this necessarily involves an increase in the introduction of single doses of "major" antigen. It would seem that "weak" antigens (and in connection with tumors they present great interest) have some advantage, since in this case it is evidently possible to carry out desensitization with smaller doses of "major" antigen (in terms of the shocking dose), and this leads to a less marked decrease of sensitivity to the "minor" antigen. However, in this case the disadvantageous aspect consists in the fact that on sensitization with small quantities of "weak" antigen the shocking dose exceeds the sensitizing 10 times. Therefore on sensitizing animals with a mixture of two weak antigens, mixed in the proportions of 50:1, it is possible to obtain a shock reaction for the "minor" antigen, if the mixture (or complex preparation) is introduced in such amounts as contain not less than 20 shocking doses of the "major" antigen. Aside from the fact that this limit is difficult to attain technically, we are forced to the conclusion that with "weak" antigens it is necessary to resort to profound desensitization.

Decrease in the sensitivity to a "minor" antigen as the result of repeated desensitization to a "major" antigen is unrelated, evidently, to the phenomenon of sensitization decay. Preliminary introduction of some other serum in place of the "major" antigen, in amounts not producing any reaction, had no effect on sensitivity to the "minor" antigen. Obviously, following repeated desensitization there is a possibility of "exhaustion" (or "weakening") of the reacting system. The results of experiments with a mixture of two weak antigens correspond to this. As was shown above, in this case desensitization to the "major" antigen was accompanied by a significantly less pronounced reaction than in experiments with a strong antigen; accordingly the sensitivity to the "minor" antigen was lowered notably less by desensitization.

The results of the experiments summarized here deserve, it seems to us, serious attention when employing the anaphylactic reaction to detect antigenic differences between normal and tumor tissues.

It should be noted that in the experiments presented here only one method of desensitization was employed, one which is used in studies on tumor antigens which appear in the literature. Other methods and rates of administering the antigen should be studied.

Therefore, the use of the anaphylactic reaction for the demonstration of components of an antigenic mixture (or components of a complex antigen), resorting to desensitization of the animals to the predominant

components, makes it necessary to carry out desensitization starting with amounts corresponding to the shocking dose, and to check completeness of desensitization (with 10 and more shocking doses).

Repeated desensitization to the predominant antigen may lead to a decrease in sensitivity of the animal to a "minor" antigen.

These data should be taken into account when applying the anaphylactic reaction to the demonstration of antigenic differences between tumor and normal tissues.

SUMMARY

Utilization of the anaphylactic reaction for distinguishing tumor antigens from antigens of normal tissues requires the preliminary investigation of 2 problems: 1) how should desensitization against "major" antigens be performed in order to be sure of its completeness, and 2) how does such desensitization affect the sensitivity of the animal to "minor" antigens? Experiments were carried out on guinea pigs which were sensitized by mixtures of two antigens (foreign serums, tissue nucleoproteins) mixed in different proportions. It was demonstrated that the desensitizing dose of a "major" antigen is several times as great as the shocking dose, and therefore that repeated injection is necessary to bring about complete desensitization. This causes a pronounced decrease of the sensitivity to the "minor" antigen. Therefore it is necessary to increase the dose of the preparation containing the mixture of antigens (for example, a tumor extract) in order to obtain a definite anaphylactic reaction. These findings should be taken into consideration in employing anaphylaxis for the demonstration of antigenic differences between tumors and normal tissues.

LITERATURE CITED

[1] Ioffe, V. I., Biull. Eksptl. Biol.i Med. 1957, 44, No. 9, 12-15.*

* Original Russian pagination. See C. B. Translation.

EXPERIMENTAL INVESTIGATION OF THE PASSAGE OF AGGLUTININS FROM MOTHER TO FETUS

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The well-known low state of immunological reactivity of newborn children suggests the problem of the role of the maternal organism in the production of the natural passive humoral immunity in the fetus and the newborn.

In the animal world there exist two principal mechanisms for transmitting antibodies from mother to offspring: their transplacental passage during the period of intrauterine development, and trophogenesis, with the milk during nursing.

A series of authors studied the possibility of transplacental passage of antibodies from mother to fetus. They obtained substantially different results in animals with the hemochorion type of placenta and in animals with other types (epithelial, endothelial, and syndesmochorion types according to Grosser's classification). It has been shown that in these latter types the intrauterine passage of anribody is not observed [3, 4, 5, 7].

In man and animals with hemochorion placentas, in which as a result of destruction of epithelium and connective tissue of the mucosa the chorionic villi of the blood vessels of the uterus come into direct contact with the maternal blood, there is established transplacental passage from the blood of the mother into the blood of the fetus of antitoxin, lysins, virus-neutralizing and complement-fixing antibodies [1, 3, 8].

As far as agglutinins are concerned, several authors deny their transplacental passage or consider it accidental [2, 6]. This led us to concern ourselves with a study of transplacental passage of agglutinins from mother to offspring.

As a model we chose dysentery, as a bacterial infection in which humoral immunity demonstrates the presence of antibodies, usually agglutinins.

In the first series of investigations the problem before us was: does passage of normal antibody agglutinins from the blood of the mother into the blood of the fetus take place and does the agglutinin level in the maternal serum affect the level in the fetal serum?

METHODS

We had available for observation healthy pregnant women who had not had dysentery or typhoid and who had not been immunized against these infections, so that the antibodies observed should belong in the category of normal antibodies.

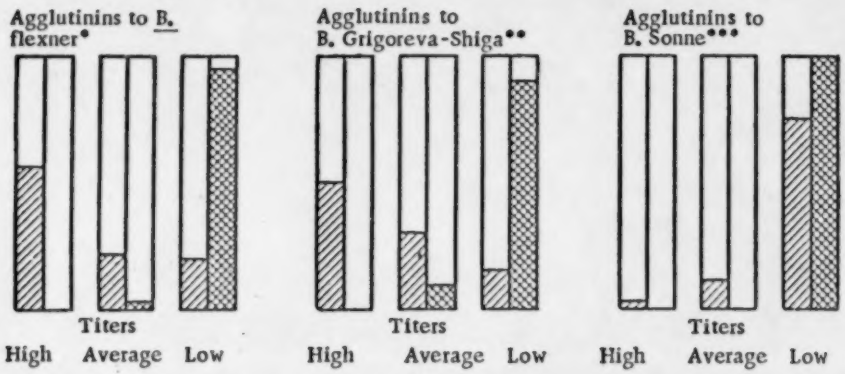
The blood for determination of antibody content was taken, following normal delivery, from the artery and vein of the umblical cord (before it was tied), from the mother's cubital vein; a sample of the retroplacental blood was also kept.

A comparative study of the agglutinating properties of the blood was carried out on 100 mothers and 100 of their newborn children; in all 400 samples were investigated (4 from each pair). We determined the

agglutinin reaction by the usual methods. However, since the sera of the newborn sometimes contained fine granules, which might be confused with finely granular agglutination, for each dilution of serum to which bacteria were added we set up a control of that dilution of serum without any bacteria.

RESULTS

On analyzing the results obtained, the fact emerges that agglutinins are encountered in the serum of the mother definitely more often and in higher titer than in the sera of the newborn. While the majority of the maternal



Correspondence of titers of agglutinins in serum of mothers and of newborn infants (in percents of the average number of positive results; slanting lines, mother; cross-hatched, child).

sera had moderately high titers (1 : 160-1 : 320) against the species of bacteria investigated by us, except sonnei, high titers were absent among the children, a medium titer (1 : 80) was observed in a single instance, and the majority of the positive reactions were obtained at low titers, not exceeding serum dilutions of 1 : 20.

The explanation of the dependence of the agglutinin content of the blood of each mother and that of her newborn child offers particular interest.

Investigation showed that with increased concentration of normal agglutinins in the maternal serum the frequency of positive reactions and the agglutinin content of the newborn went up. This permits us to state that there is a dependence between the agglutinating power of the mother's serum and that of her newborn child.

Since the method employed by us excluded the possibility of intrauterine and extrauterine active immunization of the fetus, the agglutinins to the dysentery antigen, observed in the serum of newborn infants, could have been acquired only from the mother's blood through the placenta. However, this passage cannot be regarded as a simple filtration through a semipermeable membrane; equality of antibody titers in the bloods of mother and fetus was not observed in a single instance. As a rule the antibody titer of the fetus was lower than the corresponding titer of the mother's blood.

By investigating bloods of mothers and their newborn infants we had the possibility of studying transplacental passage of normal antibodies only. Application of this method did not permit, however, observations of the dynamics of the immunological indicators before and after nursing began. Therefore a series of experiments were carried out on female rabbits subjected to a preliminary immunization, and their newborn leverets.

In setting up the experiment we were guided by the following: 1) in rabbits, as in man, the placenta produced is of the hemochorion type; 2) the leverets are born in a state of incomplete physiological maturity, which offers the possibility of observing in them the establishment of immunological reactivity.

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* Shigella flexneri.
** Shigella dysenteriae.
*** Shigella sonnei.

TABLE 1

Relation of Positive and Negative Results for Agglutination Reaction of Blood Serum of Rabbits and Their Newborn Leverets (in percents)

Species of bacterium	Results of agglutination reaction			
	Positive		Negative	
	Rabbits	Leverets	Rabbits	Leverets
Sh. flexneri	100.0	95.8	–	4.2
Sh. sonnei	81.8	43.6	18.2	56.4
Sh. dysenteriae	96.1	52.7	3.94	47.3

Transplacental passage of antibodies from the mother to the fetus in animals given preliminary immunization was studied in experiments on 26 rabbits and 71 newborn leverets.

To exclude the passage of antigen through the placenta and intrauterine active immunization of the fetus, active immunization of the rabbits chosen for the experiment was carried out with Flexner bacillus before impregnation.

The newborn leverets were taken from their dam. We took blood from them by cardiopuncture.

In order to exclude the influence of normal agglutinins on the results of our study of transplacental passage of antibodies in immunized animals, we used as controls 17 rabbits not previously immunized, and 50 of their newborn leverets.

The experiment showed that in all cases, when the antibody content of the blood of the rabbits exceeded a titer of 1 :320, agglutinins were observed in the serum of the newborn leverets, and the percent of negative results for agglutinins for Flexner bacilli, i.e., the antigen to which the dams were immunized, was only 4.2 (Table 1). The agglutinin content of the blood of the immunized animals exceeded in many cases the amount in the blood of the controls.

While in the unimmunized rabbits and their newborn leverets the agglutinins showed extremely low titers, in the immunized rabbits and their offspring the titers were significantly higher (see figure).

Analysis of our material on the agglutinating properties of serums and the agglutinin titer in each pair (female rabbit and her offspring) showed that, as in observations on newborn children, between the titers of agglutinin in the serums of the rabbits and their offspring there existed a direct dependence. This dependence was expressed more sharply in the experiment than in the observations on newborn children (Table 2).

Thus, when agglutinins were present in the blood of the female rabbits they appeared in the leverets during the period of intrauterine development. Inasmuch as immunization of the females was carried out in advance of impregnation, an active mechanism for the appearance of the antibodies in the fetus is ruled out. Therefore we may regard it as established that the appearance of agglutinins in the blood of the fetus is connected with transplacental passage from the blood of the dams.

Summarizing our observations on newborn children and newborn rabbits (first series), we must state that the hemochorion placenta is permeable to agglutinins, and only partly holds them back.

The conditions of the experiment made it possible to obrain repeated samples of blood from one and the same leveret, which permitted study of the duration of retention of the antibodies which had been acquired by them in utero.

We carried out investigations of the properties of the serum of the blood of the developing leverets the day they were born, on the 3rd, 5th, 10th, 20th, 30th, 45th, and 60th days of life. In all we examined repeatedly 50 leverets from 18 immunized dams. Study of the dynamics of retention of agglutinins permitted us to establish that newborn leverets are incapable of retaining antibodies during the course of the first 60 days of life. Between the 10th and 30th days of life in most of the leverets there begins a decrease in the original level of antibodies which were observed at the moment of birth, and by the 60th day agglutinins had disappeared from the blood of the majority of the leverets. This speaks for a passive origin for the agglutinins.

TABLE 2

Agglutinin Content in Newborn Leverets in Relation to the Magnitude of the Titers in Their Mothers (number of cases in percent)

Titers dams' serum / Titers leveret serums	Low	Medium	High and very high
1 : 10			
1 : 20	25.0		
1 : 40		9.5	
1 : 80		11.9	
1 : 160	50,0	11.9	
1 : 320	25.0	33.3	4.5
1 : 640		7.2	
1 : 1280		21.5	13.7
1 : 2560		4.7	31.8
1 : 5120			9.0
1 : 10 240			13.7
1 : 20 480			27.3
Totals	100%	100%	100%

Since from the moment of birth of a child there arises the possibility of acquiring antibodies trophogenetically, the study of the mechanism of homoral immunity at various stages of postnatal development naturally includes an investigation of the influence of nursing on the antibody content of the blood.

On the basis of cross experiments, in which immunized females nursed young born to unimmunized dams, and the reverse experiment, it was established that in rabbits, belonging to the hemochorion type of placenta, nursing did not seem to be a direct source of antibodies for the offspring, and did not exert any direct effect on humoral immunity. Natural breast feeding serves as a source of valuable and easily-assimilated proteins, fats, carbohydrates, and vitamins, promoting an increase in the natural nonspecific resistance of the newborn organism.

The reverse relations have been established for animals with other kinds of placentas (syndesmo-, epithelio-endotheliochorion), which must be considered as phylogenetically older than the hemochorion placenta. In these species of animals transplacental passage of antibodies does not go on, while the trophogenetic route seems to be the main route for the acquisition of humoral immunity in the early stages of postnatal development of the offspring. Therefore transplacental passage of antibodies from the mother to the fetus acquires significance phylogenetically as a later mechanism of production of passive humoral immunity in the newborn; in this lies its biological significance.

Summing up the results of these investigations, we believe it is possible to draw the following conclusions. The availability of antibodies in the blood of newborn at the moment of birth under conditions excluding the possibility of active formation as a result of encountering the antigenic stimulus, demonstrates their passive origin from the maternal body.

The investigations showed that in man, and animals with the hemochorion type of placenta the basic mechanism seems to be transplacental passage of antibodies; this latter is supported by the dependence of the agglutinin in the blood of the newborn on the titer in the blood of the mother. This dependence appears especially clear under experimental conditions. Transplacental passage of antibodies is supported also by the absence of trophogenetic transfer of antibodies.

SUMMARY

The agglutinin content of the blood of newborn and their mothers was studied in order to investigate the problem of the transplacental passage of antibodies.

The investigations were carried out post partum in a lying-in hospital. There were observed 100 mothers and the same number of newborn. The results were checked experimentally in female rabbits and their offspring.

It was established that transplacental passage of agglutinins from mother to fetus takes place. However, the titers of antibodies in the blood of the newborn were, as a rule, lower than the corresponding titers in the maternal blood. Consequently this passage of antibody cannot be considered to be simple filtration of antibodies through a semipermeable membrane.

LITERATURE CITED

[1] E. R. Pille, Increased Immunoreactivity in Experimental Influenza Infections, Diss.,* Moscow, 1952.

[2] H. Bourquin, Am. J. Physiol., 1922, Vol. 59, pp. 122-143.

[3] A. Kuttner and B. Ratner, Am. J. Diss. Child., 1923, Vol. 25, pp. 413-434.

[4] P. Römer, Berl. klin. Wschr., 1901, Vol. 38, No. 46, p. 1150.

[5] I. Smith and A. Holm, J. Biol. Chem., 1948, Vol. 175, No. 1, pp. 349-365.

[6] H. Rupp, Arch. f. Gyn., 1930, Vol. 143, pp. 80-145.

[7] I. Smith and R. B. Little, J. Exper. Med., 1923, Vol. 37, p. 671.

[8] J. A. Toomey, Am. J. Dis. Child, 1934, Vol. 47, pp. 521-528.

* In Russian.

ONCOLOGY

CHANGES IN CONDITIONED REFLEX ACTIVITY DURING GROWTH OF EXPERIMENTAL TUMORS, DEPENDING ON THE TYPE OF NERVOUS SYSTEM

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The dependence of the growth of tumors on the functional state of the central nervous system may now be considered as having been proven. Insufficient study has, however, been devoted to the course of the neoplastic process in animals possessing typologically different kinds of higher nervous activity, or to the effect of the neoplastic process on the conditioned reflex activity of such animals. Only a few isolated papers have been devoted to work on these problems, on white mice [4, 6] and white rats [3, 5].

The object of the present research was to study the effects of the transplantable tumor M-1 on the conditioned reflex activities of animals, taking into account the typological features of their nervous systems.

EXPERIMENTAL METHODS

The experiments were performed on white rats, the conditioned reflex activity of which had first been studied by means of L. I. Kotliarevskii's motor-alimentary method [1, 2].

A system of conditioned reflexes was elaborated in each animal, as follows: a positive conditioned reflex to red light, a positive conditioned reflex to the sound of a bell, and a negative conditioned reflex to the sound of a buzzer (differentiation to the positive conditioned reflex to the sound of a bell). Having established a dynamic stereotype in the rats, we proceeded to experiments involving change in stereotype (altered sequence of application of stimuli), extinction, and recovery of positive and negative conditioned reflexes, with the object of achieving a more precise picture of the typological characteristics of the higher nervous activity of the animals.

In determining the type of nervous system we took into account the speed of formation of positive conditioned reflexes, and their stability, the rate of establishment of differentiation , and its reinforcement, the results of modification of the dynamic stereotype, and the results of tests involving fasting for 24 hours.

An analysis of the results so obtained permitted us to segregate the experimental group of animals into their various types of higher nervous activity: strong inert, strong excitable, and weak.

To the strong inert type belonged those rats which developed a positive conditioned reflex after 10-18 associations, in which the strength of the conditioned reflex effort exerted in displacing the barrier to the feeding chamber was sufficiently great, and in which establishment of discriminatory inhibition and remodelling of the stereotype took place quickly. In most of the experiments considerable strengthening of the conditioned reflex activity took place after fasting the animals for 24 hours.

The strong excitable type of rats included those for which establishment of the conditioned reflex proceeded faster than in the preceding group, after 8-11 associations; the strength of the conditioned reflex was often greater, and discriminatory inhibition was established more slowly than in the first group, and lacked stability, and remodelling of the stereotype required a greater number of associations. Conditioned reflex activity was greatly enhanced after a 24-hour fast.

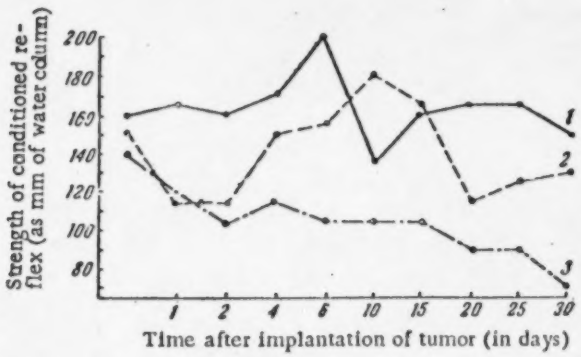
Finally, the third type of nervous system was represented by those rats in which it was difficult to establish positive or discriminatory conditioned reflexes, and in which the strength of the motor reflexes was much lower than in the first or second group. Fasting either weakened or did not affect their reflex activity.

As indicators of changes in the functional state of the nervous system we took changes in the duration of the latent period (time from application of the conditioned stimulus to appearance of the motor reflex), changes in the strength of the motor reflex (force used to displace the barrier to the feeding chamber, expressed as divisions on a scale), and the release of conditioned reflexes.

EXPERIMENTAL RESULTS

Our experiments showed that growth of the malignant tumor M-1 caused changes in the cortical and subcortical activity of the rats; these changes were more pronounced in rats of the strong types (inert and excitable).

We observed a shortening of the latent period of the reflexes in rats of the strong inert type, over a period of 8-15 days, followed by a prolongation of the latent period after growth of the tumor had begun. The strength of the motor reflex rose during the first 10 days. Discriminatory inhibition remained unchanged throughout the experiment in 4 out of 6 cases, release of reflexes being observed in 2 experiments on the 5th-9th day after implantation of the tumor. We observed the phenomenon of irradiation of inhibition in the rats of this type – application of discriminatory stimuli was followed by abolition of positive reflexes. This effect was observed on the 6th, 7th, 8th, 20th, and 24th day after implantation, in a group of 5 rats; the implant did not take in one rat. The implant began to grow on the 7th day in all five rats. The weight of the tumor amounted on the average to 58 g after 25 days.



Changes in the total magnitude of positive conditioned reflexes in rats with M-1 tumor.
1) Strong inert type; 2) strong excitable type; 3) weak type.

The latent period was shortened in a group of 5 rats of the strong excitable type, over the whole duration of the experiments. The strength of the motor reflex fell during the first 2-3 days, and then rose, to fall again at about the 20th day. Release of the differentiating reflex was observed on the 2nd-12th day after implantation of the tumor. Tumor growth began on the 6th day in 4 rats, and on the 8th-10th day in 2 rats. The average weight of the tumors was 69 g after 25 days.

Rats of the weak type did not show any such regular changes in higher nervous activity. Prolongation of the latent period was seen in 3 out of 4 rats during the first days after implantation. In one case prolongation of the latent period was observed only during the first 10 days, and was then followed by its shortening. The strength of the motor reflex varied irregularly. In 2 rats we observed abolition of positive reflexes, while discriminatory inhibition persisted over the whole period of observation. The implants began to grow on the 4th day in 2 rats, and on the 5th and 6th days in the other two. The average weight of the tumors was 82 g after 25 days.

The implants began to grow on the 5th-7th day in a group of 16 rats in which conditioned reflexes had not been formed, and the average weight of the tumors was 59 g on the 25th day.

Our results provide evidence of the significance of typological characteristics of nervous activity of rats for the development of M-1 tumor transplants. The nature of the changes in the strength of conditioned reflexes during the growth of the tumors is shown by the curves of the Figure.

In rats of the weak type, with a preponderance of inhibitory processes, the growth of the tumors began earlier, and they attained a higher weight, than in the animals of the other groups.

Conversely, in rats of the strong inert type of nervous system the positive reflexes persisted over the whole period of observation, and their strength increased. Similar changes were seen in rats of the strong excitable type of nervous system, with the difference that strengthening of the positive reflexes appeared later, and was of short duration.

It thus appears that the nature of the disturbances in nervous activity of rats during growth of the tumor is determined by the typological characteristics of the nervous system. Excitatory processes prevail in rats of the strong type of nervous system, as opposed to inhibitory processes in animals of the weak type. The prevalence of excitation in the nervous system of tumorous rats may be regarded as a factor retarding the growth of the neoplasm.

SUMMARY

Investigation conducted on rats showed that the development of malignant tumor M-1 caused changes in the cortical and subcortical nervous activity. These changes show the significance of the specific features of different types of higher nervous activity. Excitation was increased in rats of the strong type, while inhibitory processes prevailed in rats of the weak type. The tumor developed more rapidly and was of larger size in rats of the weak type of nervous system.

LITERATURE CITED

[1] L. I. Kotliarevskii, Zhur. Vysshei Nerv. Deiatel. 1, No. 5, 753-761 (1951).

[2] L. I. Kotliarevskii, Ibid., 2, No. 4, 582-591 (1952).

[3] S. I. Lebedinskaia and A. A. Solov'ev, Scientific Meeting on the Problem: "The Nervous System and the Neoplastic Processes," Texts of Papers, pp. 9-10 (Kiev, 1955).

[4] L. L. Maliugina, A. I. Mironova, V. K. Fedorov, and L. M. Shabad, Biull. Eksptl. Biol. i Med. 38, No. 9, 65 (1954).

[5] I. P. Tereshchenko, Proceedings, II Congress of Oncologists and III Congress of Roentgenologists and Radiologists of the Ukrainian SSR, pp. 46-47 (Kiev, 1956).

[6] N. M. Turkevich, Voprosy Onkol., No. 6, 64-70 (1955).

EXPERIMENTAL BIOLOGY

REGENERATION OF KIDNEYS IN MAMMALS

COMMUNICATION III. CHANGES IN THE FINE STRUCTURE OF KIDNEY DURING ITS REGENERATION

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We have shown that resection of 1/5 to 1/3 of the surviving kidney of a unilaterally nephrectomized rat is followed by an increase in the size and weight of the remaining part of the kidney, in which the structural relations are largely restored to those characteristic of the kidneys of control animals.

Of what do the changes proceeding in the injured organ, affecting its weight and dimensions, consist? The regenerative processes occurring in the injured kidney are located in the zone of injury, on the one hand, and in the residual parenchyma, not directly affected by the injury, on the other hand. These latter processes are the more pronounced, for which reason we gave priority to the elucidation of their nature and extent. With this object, we performed a detailed morphological study of the changes taking place in a rat's surviving kidney at different times after partial nephrotomy.

The results presented in the Table characterize the changes taking place in a number of structural indices of the operated kidney. All the parameters relate to transverse median sections of the kidney. So as to facilitate comparison of our results with the normal values, the first two horizontal rows of figures, given at the top of the Table, represent the results obtained on control animals at the beginning of the experiment (initial control), and at its end (terminal control).

As is evident from the Table, the number of renal corpuscles rose during the experiment, although not to the initial figure, and the relative number of renal corpuscles per mm^2 of surface was similarly not restored.

Since at the end of the experiment the total number of renal corpuscles had increased, it may be supposed that new ones had been formed. The difference between the total number of renal corpuscles on the 17th and the 89th day of the experiment is statistically significant. A certain rise in the number of renal corpuscles takes place, according to our findings, in normal rats during their growth; this is contrary to the views of some authors (Vashetko).

The mean surface area of a renal corpuscle was increased over the whole period of observation, and considerably exceeded that found in the terminal control animals, e.g., by 42% on the 89th day (Fig. 1). Hence the total surface of the renal corpuscles was found to approach closely the value found for the terminal control, over the whole period of observation. On the 89th day it exceeded this value by 9%. Although increase in the dimensions of the renal corpuscles was found from the very beginning of the experiment, and persisted throughout its duration, it is not impossible that variations in either direction may have been present at different times after the operation, but that this increase was associated with stable morphological changes in structural elements toward the end of the experiment.

Measurements of the maximum dimension of renal corpuscles showed that it had increased from 3.9 μ for normal animals to an average of 8.2 μ on the 89th day after operation.

Changes in the Structure of the Kidneys of Operated Rats

Time after operation	Number of renal corpuscles		Surface area of renal corpuscles (in μ^2)		Ratio of the surface area of the renal corpuscles to that of the cortex (as %)		Dimensions of the convoluted tubules (in μ)		
	absolute	relative (per mm² of area of the cortical zone)	of a single corpuscle	total	of a single corpuscle	all corpuscles	diameter of the tubules	height of the cells	lumen
Initial control (normal)									
	152	5.6	3741	569 697	0.014	2.10	32.4	11.3	11.4
Terminal control (normal)									
	183	4.5	4 338	793 724	0.011	1.92	35.7	10.2	11.6
Experimental									
12 hours	131	4.1	5 770	752 730	0.018	2.37	37.7	11.6	16.9
24 hours	108	3.4	5 491	565 420	0.017	1.73	38.7	12.2	15.7
2 days	143	2.7	5 685	808 680	0.011	1.55	39.8	11.9	18.5
5 "	145	2.3	5 606	817 806	0.009	1.26	40.3	11.8	16.5
11 "	136	3.4	5 127	698 238	0.012	1.68	38.8	13.2	11.2
17 "	115	2.6	6 502	746 911	0.015	1.72	44.5	12.2	17.7
23 "	126	2.4	5 984	755 712	0.012	1.46	41.0	11.9	13.8
35 "	123	2.6	5 999	735 134	0.013	1.56	42.5	11.7	19.2
47 "	123	2.5	5 742	713 274	0.012	1.47	42.0	12.6	14.3
59 "	135	2.4	5 427	728 851	0.01	1.28	40.9	11.6	15.4
71 "	140	3.0	5 482	758 351	0.012	1.65	41.6	11.5	19.6
83 "	146	2.9	5 547	806 460	0.011	1.60	46.7	12.3	18.3
89 "	139	2.8	6 178	862 285	0.012	1.71	46.5	11.6	21.9
217 "	147	2.2	6 563	950 013	0.01	1.45	42.6	12.0	19.0

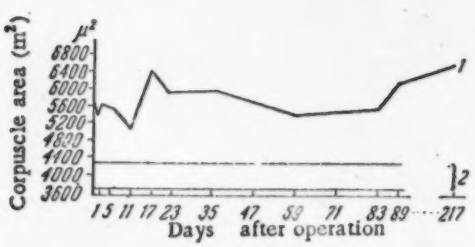

Fig. 1. Changes in the area of the renal corpuscles. Abscissae: days after operation; ordinates: surface area of the corpuscles, in square microns.
1) Operated rats; 2) initial and terminal control rats.

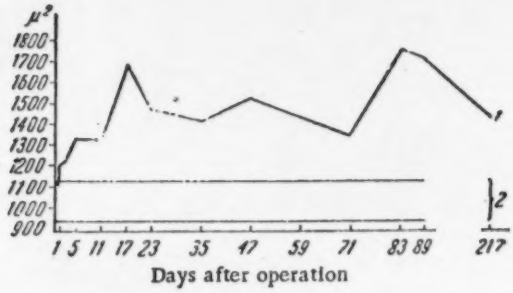

Fig. 2. Changes in the area of the wall of the convoluted tubule.
Explanation of diagram as for Fig. 1.

We counted the number of nuclei in the outer layer of the capsule of Shumlianskii, in order to ascertain whether the increase in the dimensions of this capsule was due only to hypertrophy of the cells, or to their hyperplasia.

We found that on the 89th postoperative day the capsule contained an average of 18.6 nuclei, as compared with 11.5 for normal controls. The increase in the size of the capsule can hence be ascribed to increase in the number of its constituent cells.

We also measured the distance between nuclei in the outer layer of the capsule of Shumlianskii. On the 89th postoperative day this distance amounted on the average to 13.1 μ, as compared with 8.6 μ in normal controls. It follows that not only does the number of constituent cells rise during increase in size of the capsule, but that there is also an increase in their size, i.e., they are hypertrophied.

The diameter of the cross section of the convoluted tubules rose within 12 hours of the operation, when it already exceeded that found in the terminal control. It remained higher than in the terminal control throughout the experiment, being greatest on the 83rd day (31% greater than the terminal control figure).

The height of the cells seen in cross sections of the convoluted tubules was also increased within 12 hours of the operation, and remained at a high level thereafter. It was maximum on the 11th day (29% greater than in the terminal control). This increase in height of cells is statistically significant.

The size of the lumen of the convoluted tubules was increased from the very beginning (47% greater than the control 12 hours after the operation). It was maximum on the 89th day (90% greater than the control value).

We measured the area occupied by cells on the cross section of the convoluted tubules, by subtracting from the overall cross section that part of it representing the lumen. We found that this area increased gradually from the very beginning of the experiment, and that it remained at a high level throughout. It was maximum on the 83rd day, 56% greater than for the terminal control (Fig. 1).

A count of the number of nuclei present on a cross section of a convoluted tubule showed that the figure had increased by 60% on the 89th day.

We also measured the distances between nuclei in cross sections of the convoluted tubules, so as to ascertain whether any changes had taken place in the size of the cells. On the 89th day after the operation the average distance was 9.4 μ, as compared with 7.9 μ for the control (Fig. 2), showing that cell hypertrophy had taken place here, too.

Thus, during the postoperational period dilation of the tubules had taken place, associated with increase in the number of cells and in their size.

We also studied the changes in the dimensions of the collecting tubules, in both the cortical and the medullary zones.

We found that the diameter of the collecting tubules of the cortex (as seen in cross section) had increased from the normal value of 26.7 μ to 35.4 μ on the 89th day. The diameter of the lumen had risen from 13.9 to 22.8 μ. The part of the cross sections occupied by cells had risen from 521.6 to 709.1 μ^2. The height of the cells had increased from 6.6 to 7.6 μ, but this difference is not statistically significant. The number of nuclei had risen from 5.1 to 7.6. The distance between nuclei had increased from 5.5 to 8.4 μ. This difference is of a random nature.

It thus appears that not only had dilation of the collecting tubules of the cortical zone taken place during the experimental period, but that the number of cells lining the tubules had risen. There was no significant change in the dimensions of the cells.

The diameter of the collecting tubules of the medullary zone, as seen in cross section, rose from the normal value of 25 μ to 29.7 μ on the 89th day after the operation. The lumen had increased in size from 15.1 to 18.1 μ. The area occupied by cells in the cross section had risen from 398.7 to 551.8 μ^2. The number of nuclei had risen from 4.3 to 5.4. There were no changes in the height of the cells or in the distance between nuclei. Thus the dilation of the medullary collecting tubules was associated with an increase in the number of cells lining the tubules.

As is evident from the above findings, the process of regeneration of the kidney is a complex one. The changes taking place, which affect the whole of the renal parenchyma, involve increase in the dimensions of the structural elements of the kidney (corpuscles and tubules), due chiefly to cell hyperplasia, and partly to cell hypertrophy.

Although we observed the presence of well developed regenerative processes in the damaged kidney, we wish to record that full regeneration was not achieved under the conditions of our experiment.

The normal structure of the kidney was not fully restored in our experiments. Instead, we found an increase in the dimensions of the nephrons and of their constituent parts, due chiefly to cell hyperplasia, and partly to cell hypertrophy. In addition, there was an increase in the dimensions of the intercellular spaces, in particular in the lumen of the tubules and in the volume of the corpuscles.

SUMMARY

Experiments were performed on rats. A detailed morphological analysis was carried out of changes which take place in residual part of the kidney at various periods following operation of ablation of one kidney and partial resection of the other.

The changes involve the whole parenchyma of the kidney and consist in increase of the size of the structural elements of the kidney (tubules and Malpighian bodies) caused mostly by cellular hyperplasia with their partial hypertrophy. Besides, the intercellular spaces are enlarged, particularly the tubular lumen and the cavity of Malpighian bodies.

LITERATURE CITED

N. P. Vashetko, Kharkov Med. Journal, 18, Nos. 7-11 (1914).

ANTIGENIC PROPERTIES OF ARTICULAR CARTILAGE

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The work of I. I. Mechnikov and his pupils [9, 10] not only had a decisive influence on the theory and practice of the immunity of infectious diseases, but laid the foundations for the theory of tissue and organ immunity. The work of Soviet scientists [1-8] played a large role in the subsequent development of immunology.

The situation with respect to the problem of lessening the antigenic differences of tissues and organs when they are transplanted from one person to another is definitely less satisfactory. The usual outcome of all homoplastic transplants is complete resorption of the transplants, or in the most favorable cases temporary clinical survival. Biological survival of homotransplants in the clinic has so far not been achieved because of antigenic differences of tissues and organs.

It is clear from the literature that with the aid of immunological reactions, it has been established that there are specific antigenic differences within the species, in the organs and tissues of animals and man (skin, nervous tissue, thyroid, and others). Regarding the presence of specific antigenic differences in hyaline cartilage, especially articular cartilage, one finds conflicting data. Meanwhile an answer to this question would have not only theoretical interest, but also great significance in practical medicine (homoplasty).

Considering the urgency of the problem of homoplasty of cartilage, we have been studying, since 1950, homoplasty of articular cartilage in experiments on dogs.

To eliminate the antigenic properties of articular cartilage transplants, we utilized various methods of preservation. Following storage of the transplants at 0° in serum of the recipient for 7 days (gradual cooling to 0°), biological survival was observed in two cases. But these were the usual type of experiment on transplanted tissues, where immunological reaction of the body was evaluated by the survival of the transplant. For a more precise evaluation of the immunological reaction, we decided in 1955 to investigate the antigenic properties of fresh and preserved articular cartilage, by the complement fixation (CF) reaction.

In parallel, similar experiments were set up for the determination of the specific antigenic properties of skin.

METHODS

We carried out experiments on 18 adult rabbits, which were divided on the basis of sex and weight into five groups. The immunization was carried out by the usual method.

Obtaining monospecific serum against articular cartilage and skin. Into the three rabbits of the first group we injected an extract of articular cartilage (antigen) which had been stored at 0° for 7 days, into the three rabbits of the second group we injected an extract (antigen) of fresh articular cartilage; into the three rabbits of the third group we injected an extract (antigen) of skin which had been stored at 0° for 7 days; into the six rabbits of the fourth group we injected an extract of fresh skin. Three rabbits of the fifth (control) group were not immunized.

Material for immunization was taken from young rabbits from a single litter, at the age of 40-50 days.

After removing the hair, we removed, under sterile conditions, skin and articular cartilage from the epiphsis of the femur. We cut up the material thus obtained finely with scissors, then ground it in a porcelain mortar with hypertonic salt solution (for 1 g of tissue 10 ml of a 1.7% solution of common salt). The suspension was allowed to stand 2 hours, after which we centrifuged it for 10 minutes at 2000-2500 rpm. We diluted the centrifuged liquid (antigen) with distilled water to the concentration of physiological saline. We put the extracts prepared in this way, with sterile precautions, into ampules of 3 ml and used them for immunization and for the CF reaction. Animals were selected which were nearly the same weight (2280-2317 g), and their food and quarters were of the same type.

All immunizations lasted four weeks. On the first 3 days we injected, with sterile precautions, the antigen (extract of cartilage and skin) intravenously in 1 ml amounts. After a 4-day interval we carried out a second course of immunization. On the first day we injected 1 ml intraperitoneally, and on the following 2 days 1 ml intravenously, followed by a 4-day interval. Three cycles like the second were carried out. On the 8th day after the last intravenous injection, blood was taken from all immunized and control rabbits. The serum obtained was used for the CF reaction with antigens from cartilage and skin, fresh and preserved.

It should be remarked that in order to determine the immunizing dose of the antigen we tried 2 ml of the extract (antigen) of cartilage and skin, stored at 0°, or fresh. On the first day we injected the above mentioned amount intravenously into each experimental animal. About 8-10 minutes after injection of the antigen 2 rabbits of the group died of anaphylactic shock. The others remained normal. As a result the immunizing dose was lowered to 1 ml, and the fourth group of experimental animals replenished with three new rabbits.

However, inspite of the reduction in dose of antigen, on the third week of immunization 3 rabbits of this group died of anaphylactic shock about 3-5 minutes after intravenous injection of the extract. Thus we were able to carry out a complete course of immunization to the antigen from fresh skin with only one rabbit (No. 10).

We carried out the complement fixation reaction by the usual method. By this reaction, using the antibody of the sera, we estimated the antigenic properties of the articular cartilage and skin with which we had immunized the experimental animals. For the CF reaction we diluted the antigens 10, 100, and 200 times (see table).

RESULTS

As can be seen from the table, a positive complement fixation reaction was obtained with the 1 :100 dilution in the group of animals (Experiment No. 2) which were immunized with an extract of fresh articular cartilage. Fresh articular cartilage, accordingly, possesses well-defined antigenic properties.

Articular cartilage stored at 0° for 7 days possesses distinctly weaker antigenic properties than does fresh cartilage, which is shown by the CF results in the first experiment. In this experiment, the CF reaction was positive in two rabbits at a dilution of 1 :10 in the first 15 minutes. In rabbit No. 3 the CF reaction was negative. Consequently antibody to the injected antigen was hardly formed in the blood of the animals injected with the extract of preserved articular cartilage. On the basis of this observation it may be stated that storage of articular cartilage at low temperatures lowers its antigenic properties significantly.

In the fourth experiment, where we used an extract of fresh skin for immunizing the rabbits, 5 rabbits out of 6 died of anaphylactic shock during the immunization. Only one rabbit (No. 10) survived the complete course of immunization. The CF reaction in this rabbit was negative, as may be seen from the table. On the basis of the death from anaphylactic shock of 5 rabbits in the fourth experiment, it may be suggested that fresh skin possesses pronounced antigenic properties. The negative CF reaction in rabbit No. 10 is evidently explained by a nonreactivity peculiar to this animal.

In the third experiment in which we immunized the rabbits with an extract of preserved skin, all the animals survived. However, the CF reaction was positive in all rabbits at dilutions of 1 :10 and 1 :100. This experiment showed that skin possesses pronounced antigenic properties. Storage at low temperatures perhaps lowers this property, but does not remove it completely.

The reliability of the CF results obtained in the experiment is supported by study of the sera of all the control groups. As is evident from the table, none of the sera of the control animals contained specific antibodies to any of the four antigens.

Results of Complement Fixation (CF) Reaction for the Determination of the Antigenic Properties of Articular Cartilage and Skin (fresh and stored at 0° for 7 days)

No. of expt.	Antigen used to obtain immune serum	No. of rabbit	Control without antigen		Dilution of Extract					
					1 : 10		1 : 100		1 : 200	
			Complement fixation reading after							
			15 min.	45 min.	15 min.	45 min.	15 min.	45 min.	15 min.	45 min.
1	Extract of preserved articular cartilege									
		1	h	h	+++	h	h	h	h	h
		2	h	h	++	h	h	h	h	h
		3	h	h	h	h	h	h	h	h
	Control	17,18,19	h	h	h	h	h	h	h	h
2	Extract of fresh articular cartilege		h	h	++++	++++	++++	++	h	h
		5	h	h	++	++	+++	+	h	h
		6	h	h	+++	++	++	h	h	h
	Control	17,18,19	h	h	h	h	h	h	h	h
3	Extract of preserved skin	7	h	h	++++	++++	++++	+++	h	h
		8	h	h	++++	++++	+++	++	h	h
		9	h	h	++++	++++	++++	+	h	h
	Control	17,18,19	h	h	h	h	h	h	h	h
4	Extract of fresh skin	10	h	h	h	h	h	h	h	h
	Control	17,18,19	h	h	h	h	h	h	h	h

Note. The complement fixation results are expressed in the usual way: ++++ indicates absence of hemolysis, h, complete hemolysis, +++, ++, and + intermediate degrees of hemolysis.

The decrease in the antigenic properties of cartilage as a result of storage at low temperatures suggests the possibility of further improvement in methods of preservation with the aim of weakening the antigenic properties for use in homoplastic transplants.

SUMMARY

Fresh articular cartilage possesses marked antigenic properties. Storage at low temperatures (0°C) lowers its antigenic properties considerably.

Data from complement fixation tests show that the antigenic properties of fresh and stored skin are considerably more pronounced that those of fresh articular cartilage.

LITERATURE CITED

[1] R. F. Averkina, Biull. Eksptl. Biol. i Med. 41, 2, 70-73 (1956). *

[2] A. D. Ado, Antigens as Extremely Potent Irritants of the Nervous System, **Acad. Med. Sci. USSR Press, 1953.

[3] N. N. Zhukov-Verezhnikov, Current Studies on Antigens, Proceedings XII All-Union Conference on Hygiene, Epidemiology, Microbiology and Infectious Diseases Specialists, Moscow 1948.

[4] P. F. Zdrodovskii, The Problem of Reactivity in the Study of Infection and Immunity,** Moscow 1950.

[5] L. A. Zil'ber, Fundamentals of Immunity,** Moscow, 1948.

[6] P. N. Kosiakov, Antigenic Substances of the Body and Their Significance in Biology and Medicine,** Medgiz, 1954.

[7] T. V. Lopashev, and O. G. Stroeva, Uspekhi Sovremennoi Biol. 30, 2 (1950).

[8] I. N. Maiskii, The Biological Basis of Anticancer Immunity,** Medgiz, 1955.

[9] I. I. Mechnikov, Spermatoxin and Antispermatoxin, Problems in Immunity, Selected Works, Acad. Sci. USSR Press, pp. 476-489 1951.

[10] S. Metal'nikov, and I. Strel'nikov, On the Origin of Spermatoxins, Transactions of the Saint Petersburg Biol. Lab. 12, 2-3 (1900).

[11] I. I. Khvorostukhin, Influence of Conditions of Storage of Homotransplants of Articular Cartilage on their Survival and Regeneration, "New Data on the Problem of Development of Cellular and Noncellular Forms of Living Matter,"** p. 158, Medgiz, 1954.

* Original Russian pagination. See C. B. Translation.
**In Russian.

THE EFFECT OF HORMONES AND ANTIBIOTICS ON THE REGENERATION OF CORNEAL EPITHELIUM IN RABBITS

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The purpose of the present investigation has been to study the effect of various pharmacological agents on tissue regeneration [1].

Of the hormones we used testosterone and desoxycorticosterone. Published reports on their effect on regeneration are contradictory [6, 9, 10, 12, 13, 15].

Of the antibiotics we used penicillin and biomycin. Several reports have been published to the effect that local application of high concentrations of antibiotics restrains proliferation in the region of the wound, while in low concentrations it has a stimulant effect [2, 3, 4, 8, 14]. We found no reports on the resorptive effect of antibiotics on regeneration.

METHOD

The experiments were carried out on adult male rabbits. We used the method of Ponomareva-Astrakhantseva [5], which consists of scarifying the corneal epithelium, staining it with fluorescein, and measuring the extent of the damaged area.

To start with we measured the average time required for the corneal epithelium to regenerate after a scratch 4-5 mm long had been made in it (and a subcutaneous injection of isotonic saline was given). This time we found to be 6.42 ± 0.64 hours.

After this we found the effect on regeneration of the substances to be investigated when injections of them were given 20 minutes before or 2 and 4 hours after making the wound. The dose to be given was calculated from the mean therapeutic dose for a human adult. This was calculated from the formula: dose/kg weight × $\times \sqrt[3]{\text{the body weight}}$ = Constant, [7, 11].

RESULTS

Experiments with Testosterone. This was injected in doses of 0.001 and 0.005 g per kg weight.

A dose of 0.001 g/kg, corresponding to a single dose for man of 0.005 g/kg of testosterone had no definite effect on the time taken for the corneal epithelium to regenerate. A dose of 0.005 g/kg was therefore tried. This dose is equivalent to one for man of 0.05 g/kg.

When the 0.005 g/kg injection was given before causing the damage, the repair time was shortened by 0.72 hours, when given 2 and 4 hours after the damage, the reduction was 0.59 and 1.08 hours respectively.

Experiments with Desoxycorticosterone. Desoxycorticosterone acetate, supplied by the firm Organon, was given as a subcutaneous injection of 0.00025 g/kg. When the injection was given before inflicting the damage the

repair time was shortened by 0.86 hours, and when injected 2 and 4 hours after the damage the reduction was 1.14 and 1.09 hours respectively.

Thus, in our experiments, testosterone in doses of 0.005 g/kg and desoxycorticosterone in doses of 0.00025 g/kg increased the rate of regeneration of the corneal epithelium (Table 1).

TABLE 1

Increase in Rate of Regeneration of the Cornea Caused by Sex Hormones (in hours)

Substance	Regeneration when hormone was injected		
	Before damage	2 hours after damage	4 hours after damage
Testosterone	0.72	0.59	1.08
Desoxycorticosterone	0.86	1.14	1.09

Experiments with Penicillin. We investigated the effect of both amorphous and crystalline penicillin. Injections of 6,000 units/kg of both preparations were given intramuscularly.

When the injections were given before the damage, the amorphous penicillin advanced regeneration by 1.15 hours, and the crystalline by 1.09 hours. When given 2 hours after damage, the reduction in time resulting from the amorphous penicillin was 2.13 hours, and that from the crystalline, 2.15 hours.

Experiments with Biomycin. 10,000 units/kg were injected into the stomach. When given before damage healing time was reduced by 0.47 hours, and when given after 2 hours – by 0.72 hours.

Thus, in these experiments, both penicillin and biomycin stimulated regeneration of the corneal epithelium of the rabbit. This effect was greatest in the case of penicillin. Both amorphous and crystalline varieties had the same effect (Table 2).

TABLE 2

Increase in Rate of Regeneration of the Cornea Caused by Antibiotics

Substance	Regeneration time (in hours) when injections given	
	Before damage	2 hours after damage
Amorphous penicillin	1.15	2.13
Crystalline penicillin	1.09	2.15
Biomycin	0.47	0.72

The published reports on the effect of antibiotics on the growth of young animals and on metabolic rates, and our results, suggest that the therapeutic effect of antibiotics on wound healing is not only due to their bacterial action but also to their effect on the body itself.

In these experiments both hormones and antibiotics accelerate regeneration of rabbit corneal epithelium.

SUMMARY

Testosterone (0.005 per kilogram of body weight), desoxycorticosterone (0.00025 per kg), penicillin (6,000 units per kg) and biomycin (10,000 units per kg) were the preparations administered in this experiment. Their stimulating effect on the healing of the corneal defects in rabbits was established. The preparation was introduced before the injury was inflicted, or directly after it (in 2 to 4 hours).

LITERATURE CITED

[1] G. S. Alekseeva, Effect of Various Pharmacological Agents on Regeneration of Rabbit Corneal Epithelium,* Dissertation, 1956.

* In Russian.

[2] G. R. Dambite, Clinical and experimental observations on the healing of Ulcus Serpens Corneae by Sulfanilamide Preparations and by Penicillin,* Author's abstract of Dissertation, 1951.

[3] G. K. Mironich, Voenno-med. zhurn. 3, 27-32 (1953).

[4] E. A. Timofeevskaia, Biull. Eksptl. Biol. i Med. 10, 313-315 (1951).

[5] L. Z. Ponomareva-Astrakhantseva, Farmakol. i Toksikol. 13, 4, 54-55 (1950).

[6] N. A. Shereshevskii, Nov. med. 12, 3-5 (1949).

[7] H. C. Bazett and W. H. Erb, J. Pharm. Exp. Therap. 1933, Vol. 49, No. 3, pp. 352-360.

[8] O. Bucher, Schweiz. Med. Wchschr. 1946, Vol. 76, pp. 16-17, 375-378.

[9] C. Covallero, et. al., Arch. internat. pharmacodyn. 1951, Vol. 86, pp. 43-51.

[10] R. L. Howes, J. internat. chir. Brux. 1953, No. 13, 2, pp. 127-133.

[11] H. J. Klarc, Handbuch der exper. Pharmac. 1937, No. 4, pp. 166-167.

[12] C. L. Pirani, et. al., J. Exper. Med. 1951, 93, 3, 217-218.

[13] U. Principe and G. Bellucci, Sperimentale, 1952, 102, 8, 9, 10.

[14] G. K. Smelser, Am. J. opth. 1946, Vol. 29, No. 5, pp. 541-551.

[15] M. Taubenhaus and G. D. Amromin, Endocrinology 1949, Vol. 44, No. 4, pp. 359-367.

* In Russian.

MORPHOLOGY AND PATHOMORPHOLOGY

EFFECT OF LEAD NITRATE ON THE SUPRAVITAL STAINING OF CELLS OF POIKILOTHERMS

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The present paper is devoted to a study of the effect of lead nitrate poisoning of poikilotherms, using a method involving supravital staining of the whole organism.

Supravital staining techniques are widely applied in cytology and physiology [9]. In most cases they are applied to isolated organs, and the resulting findings cannot always be taken as valid for the organism as a whole.

Various methods have been used in the study of lead poisoning, but we have been unable to find any references to the application of supravital staining. This method, however, has many advantages over the usual histological methods, since it permits of the study of reversible changes, does not require the use of powerfully acting fixatives, and permits the observation of changes in the physiological state of cells.

D. N. Nasonov [12] and P. V. Makarov [5] introduced neutral red into the gastrointestinal tract of animals, using a specially designed instrument for this purpose. A. A. Braun and M. F. Ivanov [1] injected the dye into the body cavity. The most useful supravital stain is neutral red [7, 8], which stains most tissues, including nervous tissue elements. E. V. Gubler [4], N. I. Grebenskaia [3], A. A. Savich [10], and E. M. Gramenitskii [2] have applied supravital staining of the whole organism to the study of the nervous system of a variety of animals.

EXPERIMENTAL METHODS

Autumn and winter frogs (males) were given injections into the body cavity of 1% aqueous neutral red solutions, at dosage levels of 0.3-0.5 mg of dye per g weight of animal. Over 300 experiments were performed in all. In most of the experiments, the organs under study were removed an hour after the injection of the dye, and were subjected to microscopic examination. Such studies of surviving tissues were conducted for the central nervous system, spinal ganglia, sympathetic nerve chains, liver, and kidneys.

We selected sensory nerve cells of the spinal ganglia for study because they represent links of reflex arcs, an analysis of the activity of which is relatively simple and readily performed. A study of the sympathetic nerve cells permits the assessment of the state of the autonomic nervous system, which is known to suffer injury in lead poisoning; cellular elements of the liver and kidneys were studied because these organs are involved in the accumulation and excretion of lead.

The techniques of isolation of spinal ganglia and sympathetic chains, and of preparation of surviving liver and kidney tissues for microscopic examination, have been described in the literature [2, 6, 11]. Observation of individual animals was conducted over several days. Injection of the stain had no perceptible effect on the condition of the animals. The distribution of the stain over all the organs of the body was assessed by means of colorimetric and microscopic examination.

EXPERIMENTAL RESULTS

Figure 1,a illustrates supravital staining of nerve cells of sympathetic chain ganglia 1 hour after injection of dye. Numerous, well defined granules of dye appear in the cytoplasm. These granules are distributed mostly

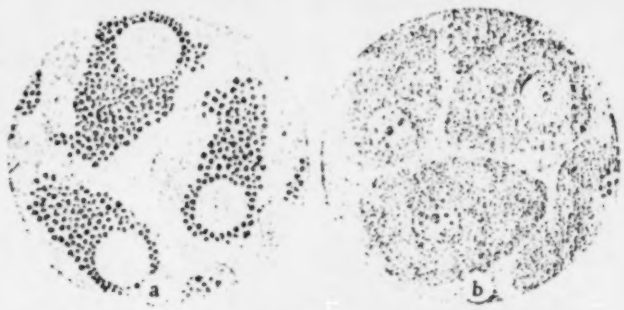

Fig. 1. Supravital staining of sympathetic nerve cells 1 hour after injection of neutral red and lead nitrate.
a) Control experiment; b) after simultaneous injection of neutral red and lead nitrate.

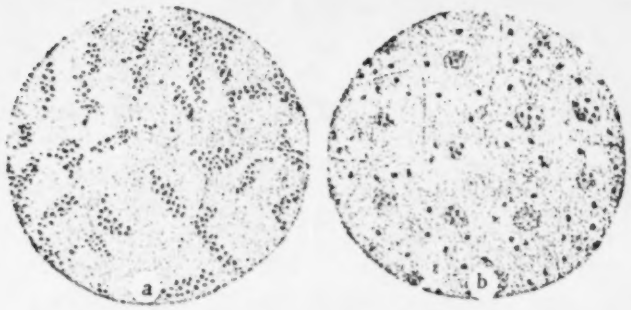

Fig. 2. Supravital staining of liver cells 1 hour after injection of neutral red and lead nitrate.
Explanation of Figure as for Fig. 1.

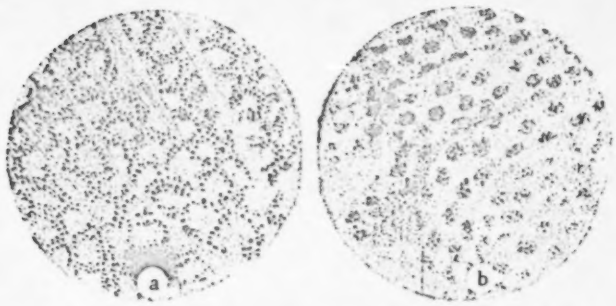

Fig. 3. Supravital staining of cells of the renal tubules 1 hour after injection of neutral red.

in the perinuclear region, and are never encountered in the neurite; they occupy a limited zone of the cytoplasm, corresponding with the intracellular apparatus. The neutral red granules have an irregular, angular shape, and are of different sizes. They are usually of a yellowish red color. The cytoplasm itself, and the nucleus, remain unstained. The distribution of the dye in the cytoplasm of uninjured spinal ganglion cells is similar,

with the sole difference that the cytoplasm takes on a light orange tinge, and the granules are smaller. No change in the reflex activity of the animals could be observed in the control experiments, showing that the supravital staining of the cells described is characteristic of normally functioning cells.

Large, deep red granules of dye were sparsely distributed in separated groups at the periphery of liver cells bordering the bile ducts. Dark granules of pigment may be encountered among the dye granules. The cell granules are not clearly defined. The nucleus and nucleolus are not stained (Fig. 2,a). The cells of the renal tubules are usually viewed from a polar aspect, for which reason the granules of neutral red are seen at the peripheries of the cytoplasm. They have a reddish tinge, and they are distributed along the cell boundary (Fig, 3,a).

It thus appears that in the intact organism, with uninjured, normally functioning cells, neutral red is deposited in the form of granules.

The frogs were poisoned by injection of aqueous solutions of lead nitrate, at dosage levels of 1-10 mg per g. The dye and the lead salt were injected simultaneously, but into different parts of the body, viz., the lead salt was injected into the dorsal lymph sac, and the dye into the body cavity.

Microscopic examination showed that administration of lead nitrate affected the nature of the supravital staining of the cells. With rising dosages of lead nitrate, diffuse staining of the cytoplasm appears, and the nucleus and nucleolus become visible, while the process of granule formation is suppressed, i.e., the complex of changes characteristic of paranecrosis supervenes [9].

Paranecrotic changes were seen in liver cells at dosages of 3 mg/g. The cell nuclei were stained, and granular structures appeared in them, while the cytoplasm assumed a uniform, diffuse, bluish-red coloration, and there was a marked fall in the number of granules (Fig. 2,b and Table).

Supravital Staining of Cells 1 Hour after Simultaneous Injection of Neutral Red and Lead Nitrate to Male Frogs (mean results of 50 experiments)

Dose of lead nitrate (mg/g)	Cell	Supravital staining		
		granules	nucleus	cytoplasm
3	Sensory nerve	+	0	0
	Sympathetic nerve	+	0	0
	Liver	0	+	+
	Renal tubules	+	0	0
7	Sensory nerve	+	0	0
	Sympathetic nerve	0	+	+
	Liver	0	+	+
	Renal tubules	+	0	0
10	Sensory nerve	+	0	0
	Sympathetic nerve	0	+	+
	Liver	0	+	+
	Renal tubules	0	+	+

Note: + presence of staining, 0 absence of staining.

The dose of lead nitrate needed in order to cause paranecrosis of the cells of the sympathetic chain was 7 mg/g. At this dosage, the cytoplasm was stained a uniform bluish-red color. Granules of dye were absent from the cytoplasm. The cell nuclei became clearly distinguishable owing to the diffuse pale pink staining of karyoplasm. The nucleoli were also stained (see Fig. 1,b and Table).

Raising the dosage level to 10 mg/g caused changes in the staining of the cells of the renal tubules. The cytoplasm of these cells was also diffusely stained a light bluish-red color, the process of granule formation was suppressed, and the nucleus, containing granular structures, became visible (Fig, 3,b). The diffuse staining of these cells, characteristic of the paranecrotic state, should be ascribed to the action of the lead salt, since the dye alone never gave rise to paranectotic changes in the given cells.

The nature of the staining of the sensory cells of spinal ganglia was not affected by even very high dosage levels of lead nitrate, in spite of the complete abolition of reflex excitability of the animals towards the end of the experiments. The absence of the paranecrotic type of staining in the nerve cells of the sensory ganglia cannot have been due to shortcomings in the methods used. The presence of granules in these cells shows that the dye penetrated into them in sufficient amount. Evidently lead nitrate, in causing irreversible loss of reflex activity, attacks synaptic links in the first place, without affecting the morphology of the individual cells of the nervous system. The work of P. V. Makarov [6] supports the possibility of such an interpretation of the mechanism of action of lead. In his studies on generalized narcosis of the nervous type, he showed that reflex excitability is abolished while the sensory nerve cells still retain the granular type of staining.

Our experimental material demonstrates the possibility of applying supravital staining of the whole organism with the basic dye neutral red, for the study of poisoning.

SUMMARY

A modified method of neutral red staining of the organs of cold-blooded animals in situ was used and the effect of lead nitrate on various cells was studied. The following threshold paranecrotic doses were established for this salt: 3 mg per g of body weight of liver cells, 7 mg per g for cells of kidney tubules and 10 mg per g for sympathetic nerve cells. Paranecrotic changes were not revealed in the sensory nerve cells of the spinal ganglia, although reflex activity of the laboratory animals was depressed. The modified method of staining in vivo is recommended for use in toxicology.

LITERATURE CITED

[1] A. A. Braun and M. F. Ivanov, Arkh. Anat. Gistol. i Embriol. 12, No. 1, 3-26 (1933).

[2] E. M. Gramenitskii, Biull. Eksptl. Biol. i Med. 34, No. 2, 76-78 (1952).

[3] N. I. Grebenskaia, "Analysis of the Effect of Carbon Monoxide on the Organism, using Supravital Staining Techniques,"* Thesis, Leningrad, 1951.

[4] E. V. Gubler, "Supravital Deposition of Neutral Red in Brain and Muscle under Conditions of Oxygen Deprivation,"* Thesis, Leningrad, 1949.

[5] P. V. Makarov, Arkh. Anat., Gistol., i Embriol. 13, No. 1, 41-60 (1934).

[6] P. V. Makarov, ibid., 19, Nos. 1-2, 5-104 (1938).

[7] M. E. Mandel'shtam, Arkh. Biol. Nauk 53, Nos. 2-3, 131-142 (1939).

[8] M. E. Mandel'shtam, ibid. 58, No. 2, 125-134 (1940).

[9] D. N. Nasonov and V. Ia. Aleksandrov, "Reaction of Living Matter to External Factors,"* Moscow, 1940.

[10] A. A. Savich, "Variations in Sensitivity to Narcotics in Philogenesis and Ontogenesis,"* Thesis, Leningrad, 1951.

[11] E. Ia. Smoilovskaia, Arkh. Anat. Gistol. i Embriol. 19, Nos. 1-2, 105-115 (1938).

[12] D. N. Nasonov, Protoplasma, 1932, Vol. 15, pp. 239-267.

* In Russian.

METHODS

A THERMOELECTRIC METHOD FOR STUDYING THE CEREBRAL CIRCULATION IN UNANESTHETIZED DOGS

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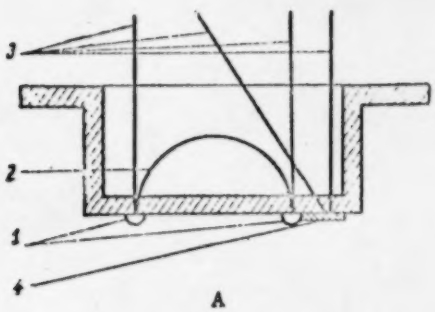
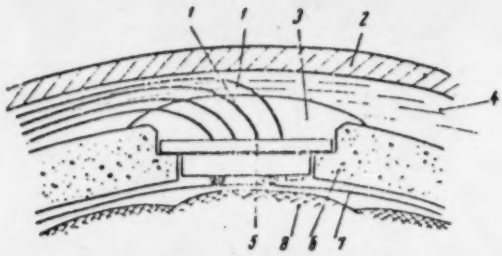
Cerebral circulation in animals has been studied chiefly in acute preparations under general anesthesia. This type of experiment cannot be relied upon to give a true picture of changes in the cerebral circulation. It is therefore very important to develop a method which makes it possible to observe the circulation in the brain under normal conditions.

In deciding upon a method, the following points must be considered: 1) the method must not disturb the normal blood and fluid circulation, 2) there must be no trauma to the brain and 3) the cranium must remain hermetically sealed.



A

Fig. 1A. Diagram of thermoelectrode. 1) Thermocouple junctions; 2) constantan; 3) copper leads; 4) nickel disk.



B

Fig. 1B. The thermoelectrode in the skull (diagrammatic). 1) Leads; 2) skin; 3) filling of dental cement; 4) muscles; 5) thermoelectrode; 6) bone of skull; 7) dura mater; 8) brain.

The thermoelectric method satisfies these conditions best, since there is no damage to the tissues, no resistance to blood flow, and the thermoelectrode can be inserted so that the cranium remains hermetically sealed.

In view of these considerations, we implanted the thermoelectrode in the intact dura. Control experiments showed that in this position, the thermoelectrode registers changes in blood flow in both the dura and the pia mater. The thermoelectrode is so placed that it is possible to measure changes in the blood flow in the meninges immediately under it.

To record changes in venous flow, the electrode was placed on the dura over the superior sagittal sinus.

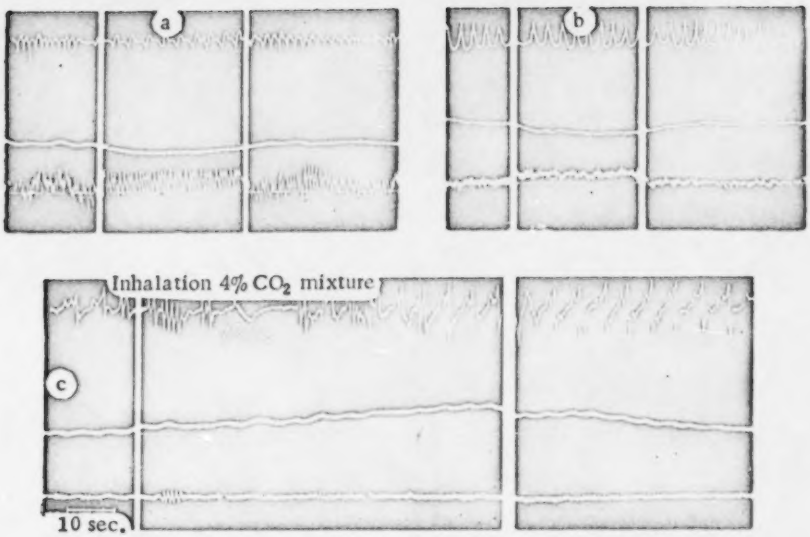


Fig. 2. Effect of compressing one of the common carotid arteries on rate of flow of blood (a) in superior sagittal sinus and (b) in the cerebral meninges. (c) Effect of breathing 4% CO_2 on rate of blood flow in the meninges.
Curves from above downwards; respiration, rate of blood flow in cerebral meninges (b, c), rate of flow of blood in sagittal sinus (a), blood pressure. Dotted line – initial rate of blood flow.

As investigations in this laboratory have shown, the thermoelectric method can be used only to make a qualitative estimate of increase or decrease of blood flow. To measure cerebral circulation in unanesthetized animals, a special flat thermoelectrode was used, as proposed by M. E. Marshak [1], this being a modification of A. Noyons' method [2]. The flat thermoelectrode consisted of a round hollow flat bottomed vessel with flanged edges made of plexiglass. The width of the edge was 1 mm, the diameter of the vessel – 5-6 mm, height – 1.5-2 mm. The copper-constantan junctions and the nickel disk for heating one of them were mounted at the bottom of the vessel. The leads from the junctions and the nickel disk were taken out through the cup and fixed to a flanged edge. The cavity of the vessel with the leads from the junctions and the nickel disk was filled with liquid plexiglass.

To apply the thermoelectrode to the skull an opening was cut which precisely fitted the thermoelectrode (Fig. 1B). The thermoelectrode was then placed in the aperature, with the junctions close up against the dura, and the edge lying in an area countersunk into the bond (see Fig. 1B). The space over the thermoelectrode was filled with a dental cement. For the cement to hold the thermoelectrode was filled with a dental cement. For the cement to hold the thermoelectrode firmly, the bond at the site of operation must be dry. Therefore during the operation it is essential to arrest hemorrhage from the bone, and to be meticulous in drying the edge of the hole.

The leads from the thermoelectrode are covered in polyvinyl chloride insulation, carried under the skin and brought out to the surface 5-6 cm below the occipital protuberance. After the operation a special collar was fixed to the neck to guard the leads from damage. Four animals with inserted electrodes were kept under constant observation for 7-19 days after the operation. In one of them the thermoelectrode was inserted with the junctions in the parietal region and in the region of the superior sagittal sinus.

We found that the thermoelectrodes were adequately sensitive to changes in cerebral circulation (Fig. 2). Comparison of changes in the cerebral circulation in the superior sagittal sinus and in the parietal region showed that in both cases the change is in the same direction for a given stimulus (Fig. 2, a and b).

SUMMARY

The use of a thermoelectric method for the study of blood supply to the brain in unanesthetized dogs is described. Modified flat Noyons' thermoelectrodes were placed on the intact dura mater in the parietal region or in the area of the superior sagittal sinus. 4 dogs with such thermoelectrodes were under continuous observation from 7 to 19 days following the operation. It was established by these experiments that in these conditions the thermoelectrodes are quite sensitive to the changes of the blood flow of the brain. These changes in the superior sagittal sinus and in the parietal region are similar when the same influence is exerted.

LITERATURE CITED

[1] M. E. Marshak, Biull. Eksptl. Biol. i Med. 43, 1, 121-122 (1957).*

[2] A. K. M. Noyons, N. V. Westernrijk and J. Jongbloed, Arch. Neer. de Physiol. 1936, Vol. 21, pp. 377-432.

* Original Russian pagination. See C. B. Translation.

BALLISTOCARDIOGRAM RECORDING IN ACUTE EXPERIMENTS ON DOGS

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Present views on the origin of the ballistocardiogram (BCG) and on the reasons for its changes are based on the work of I. Starr and his co-workers [9] on cadavers, and on recordings from healthy and sick human patients subjected to various pharmacological, operative, and other treatments. Until now only a small amount of work has been done on animals, although all the work on dogs suggests that this animal is highly suitable as the BCG and its variations are very similar to those in man. This conclusion is all the more convincing in view of the fact that various investigators have used various types of ballistocardiograph, and have used various methods of attaching it to the animals. Of these methods the most rational appear to be those in which a reliable contact is made between the transducer and the bony skeleton of the animal. This method eliminates relative movement between the transducer and the body which always occurs when the former is fixed to the soft tissues, and gives a more reliable trace.

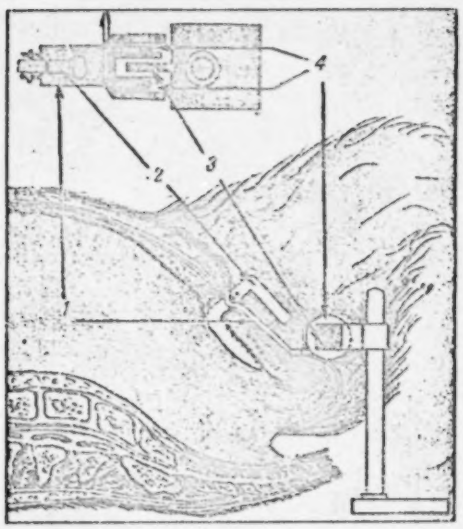

Fig. 1. Attachment of transducer for recording ballistocardiogram.
1) Duralumin platform; 2) clamp; 3) magnet; 4) coil (in the lower drawing the front coil is not shown).

However the suggestion of T. D. Darby and his co-workers [6] of fixing the transducer to the transverse processes T_6-L_5 is not convenient experimentally, as the animal must then be placed on its belly, and this complicates or even excludes the possibility of manipulation inside the thorax. W. H. Frederick, H. D. Thomas and others [8] recorded the BCG using a piezoelectric recorder with air transmission and applying the sensitive portion under a constant pressure of 5 mm of mercury to the parieto-occipital region. Both methods require the head to be fixed to the table, and this may cause distortion of the trace, particularly when it is recorded from the head.

The method of fixing V. Dock's electromagnetic transducer to the hind leg which was used by P. Cossio and his co-workers [4, 5], cannot claim to reproduce the oscillations of the body accurately as movement of the hip joint is not excluded.

The transducer we have constructed for recording the BCG in acute experiments consists of a platform which can be clamped to the canine pubis (Fig. 1). The surface of the platform which lies up against the pubis has a longitudinal groove cut in it with toothed edges which correspond with the pubic crest. The end of the

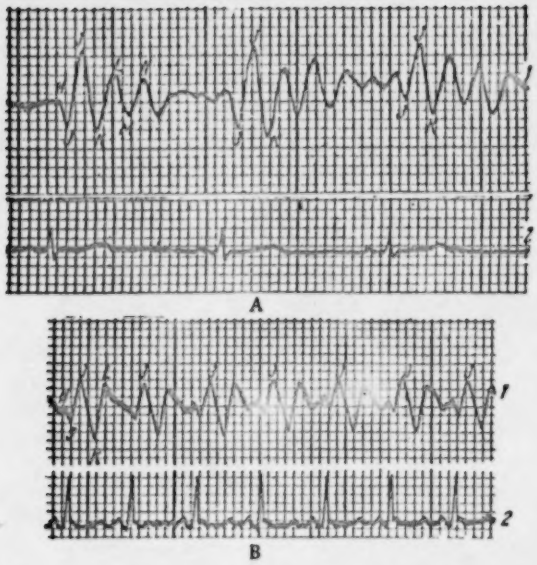

Fig. 2. (A) Human and (B) canine ballistocardiogram. For technical reasons the ECG waves are retarded by 0.04 seconds with respect to the BCG. Paper speed 40 mm/second.
Curves from above downward : ballistocardiogram of healthy male subject aged 32, electrocardiogram of the same subject; ballistocardiogram of dog with opened thoracic cavity, electrocardiogram to the same animal.

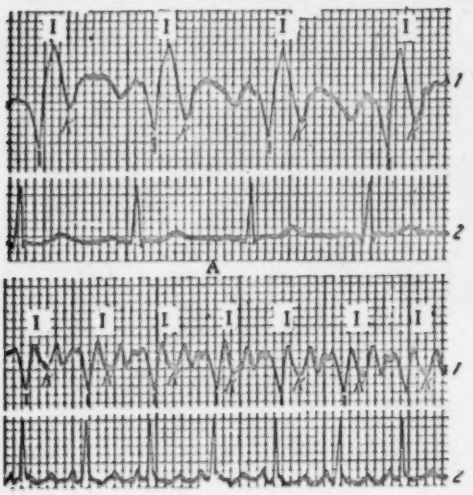

Fig. 3. (A)Human and (B) canine ballistocardiogram. For technical reasons the ECG waves are retarded by 0.04 seconds with respect to the BCG. Paper speed 40 mm/second.
Curves from above downward : ballistocardiogram of patient S, diagnosis: coarctation of the aorta, electrocardiogram of the same subject; ballistocardiogram of dog with experimental constriction of the ascending aorta, electrocardiogram of the same dog. The ballistocardiogram shows clearly a deepening of the I wave and a shortening of the K wave.

clamp which bears against the inner surface of the symphysis pubis is provided with teeth to prevent slipping. Further support is obtained from lugs in the clamp which engage with hooks on the platform. The former can be placed at any convenient level. These parts are made from sheet duralumin 5 and 10 mm thick. Any convenient system for transforming mechanical impulses into electrical ones may be mounted on the platform. We used W.Dock's [1, 7] electromagnetic system, which we had also used in our investigations on human subjects [2, 3]. The coils were fixed to the platform and the magnet to a stand.

Before placing the transducer in position, an incision is made along the linea alba of the belly. This extends from a point 4-5 cm anterior to the superior edge of the pubis to the superior apart of the perineum. The incision is made through the skin, subcutaneous tissue, superficial fascia and the aponeurosis. The recti muscles are separated without cutting, and the abdominal cavity is not opened. The pubic region is freed from fatty tissue, and in males the corpora cavernosa are displaced to one side and ligated. The parietal mesentery is separated with the finger from the inner surface of the pubis and the toothed end of the clamp is introduced between the two. The platform with the coils is screwed up tight against the pubis. The operation takes very little time and there is a negligible loss of blood.

The animal was untied and placed on its back in a box with sand or on a firm table. Sandbags were placed on either side to maintain the position. The head was fixed in the same way. The experiments were carried out in a room with a stone floor.

For anesthesia we gave a preliminary injection of 0.5 ml per kg of a 2% solution of morphine hydrochloride followed by intravenous barbamyl*; this gave a good muscular relaxation and deep anesthesia with quiet slow breathing.

In experiments in which the thoracic cavity was opened artificial respiration with slight hyperventilation was applied. After the apparatus was switched off there were no respiratory movements from the animal for more than 1 minute, which time was fully adequate for the recording of the BCG. When the thoracic cavity was not opened, the BCG was recorded during normal respiration, the frequency of which varied from 6 to 12 per minute.

To record the BCG trace the leads from the coils may be connected to the input of the amplifier of any suitable recording apparatus.

The advantages of this method consist firstly in the firm attachment of the transducer to the skeleton by means of a simple operation which takes very little time, and secondly in the position of the dog which allows free access for any operative procedures.

Figure 2 shows how close is the relation between the BCG of a dog obtained by this method and the curve for a human subject. Figure 3 shows how the same changes in the curves occur under the same condition affecting blood flow.

A full description of the ballistocardiogram in intact dogs, as well as its changes in various experimental conditions will be given in a subsequent communication.

SUMMARY

The construction of the ballistocardiogram described in this article provides reliable attachment to the movable part of the electromagnetic pickup on the dog's symphysis pubis. Thus the precise reproduction of the oscillations of its body is obtained. The operation which is required for this is very simple and does not take much time.

Position of the animal on the back gives wide possibilities for surgical interventions. Stability of this position is achieved by placing sand bags around the body. Intravenous barbamyl anesthesia caused deep sleep, slow respiration and good relaxation of muscles. In surgical interventions on the thorax, ballistocardiogram records were taken during temporary stop of artificial respiration. Moderate hyperventilation necessary for ballistocardiography excluded the possibility of spontaneous respiratory motions during the experiments. Similarity of ballistocardiograms of dogs with those of human beings gives the opportunity of studying some aspects of ballistocardiography on dogs.

* Russian trade name.

LITERATURE CITED

[1] W. Dock, G. Mandelbaum and R. Mandelbaum, Ballistocardiography,* Moscow 1956.

[2] V. V. Parin, Klin. med. 34, 36, 12-24 (1956).

[3] V. V. Parin and A. V. Mareev, Atherosclerosis and Coronary Insufficiency,* Moscow. 235-248 (1956).

[4] P. Cossio, J. A. Berreta, H. E. Mosso and I. Perianes, Cardiologia 1954, Vol. 24, No. 6, pp. 372-377.

[5] P. Cossio, J. A. Berreta and H. E. Mosso, Amer. Heart. J. 1955, Vol. 49, No. 1, pp. 72-76.

[6] Th. D. Darby, L. I. Goldberg , P. C. Gazes and S. R. Arbeit, Proc. Soc. Exper. Biol. and Med. 1954, Vol. 86, No. 4, pp. 673-676.

[7] W. Dock, Tr. A. Am. physicians, 1949, Vol. 62, p. 148.

[8] W. H. Frederick, H. D. Thomas, J. L. Knowles W. T. Tucker and E. E. Eddlman, Am. Heart. J. 1955, Vol. 50, No. 3, pp. 416-423.

[9] I. Starr, O. Horwitz, R. L. Mayock and E. B. Krumbhaar, Circulation 1950, Vol. 1, pp. 1073-1096.

[10] H. D. Thomas, W. H. Frederick, J. L. Knowles, T. J. Reeves, R. Papas and E. E. Eddlman, Am. Heart J. 1955, Vol. 50, No. 3, pp. 424-434.

* In Russian.

DROP COUNTER, PHOTOCELL, AMPLIFIER, AND COUNTING DOWN CIRCUIT FOR MEASUREMENT OF BLOOD FLOW WITH PEN-RECORDER

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Two types of counter have been used for measuring blood flow by means of a pen-recorder [3]. In the first of these, the blood drops close contacts inside a chamber [4], and in the second they interrupt a light beam which passes through the transparent dropping chamber onto the cathode of a gas filled photocell [5, 6]. However these counters are not satisfactory.

In order to prevent the formation of fibrin on the contacts of the first type of counter, the voltage has to be considerably reduced and the amplification correspondingly increased. During the measurement, the inner wall of the dropping chamber and the contacts become covered with a film of moisture and small fragments of blood, and this causes the contacts to close, and it becomes necessary to interrupt the experiment frequently to prevent this happening. On account of the size of the photocell (in the counter we used the photocell was a TsG-3) and the comparatively large lamp and focusing system, the second type of counter was very large, and it was not easy to use because of the necessity of directing the drops of blood rather accurately through the narrow light beam inside the dropping chamber.

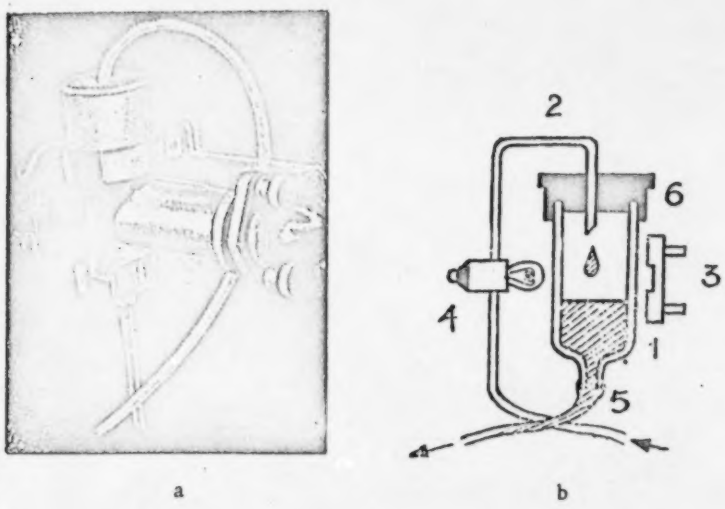

Fig. 1. Photograph and drawing of counter.

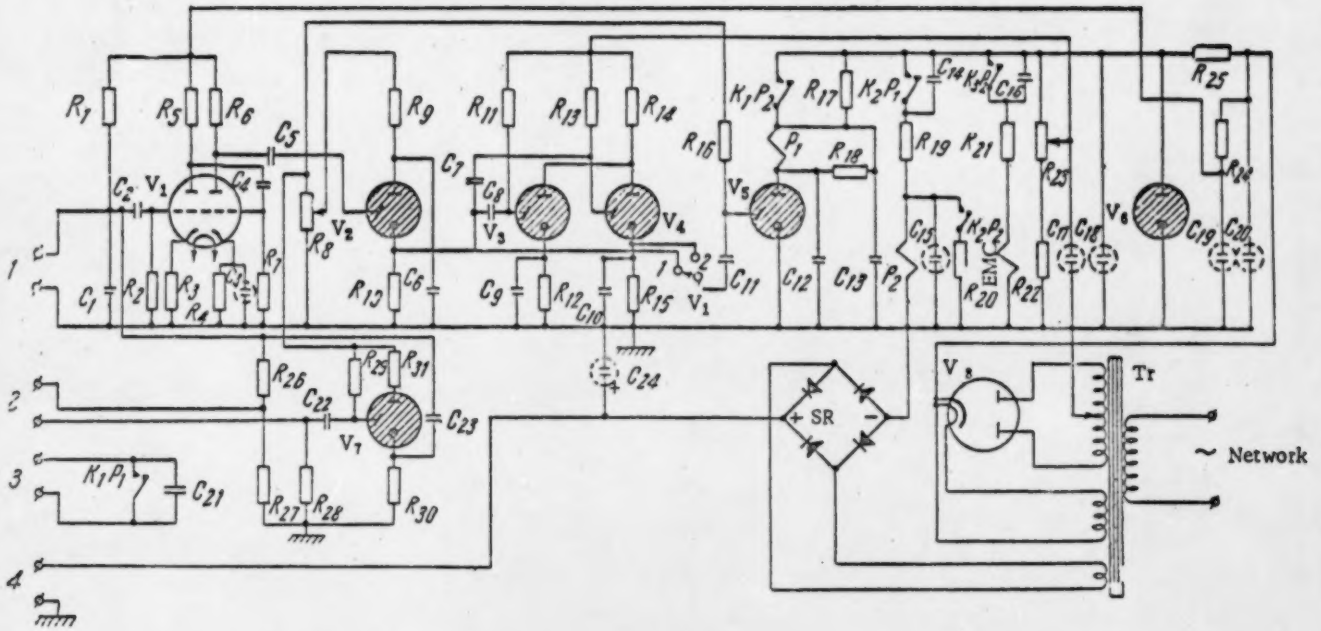

Fig. 2. Circuit of the amplifier.

Resistances R_1, R_9, R_{10}, R_{11}, R_{31} – 1 kohms; R_3, R_4 – 1.5 kohms; R_{23} – 3.3 kohms; R_{21} –5 kohms; R_{25} – 6 kohms; R_{22} – 6.8 kohms; R_{20} – 7.5 kohms; R_{17}, R_{24}, R_{30} – 10 kohms; R_{16} – 15 kohms; R_{19} – 18-22 kohms (selected): R_{29} – 30 kohms; R_{18} – 39 k ohms; R_{13} – 47 kohms; R_5, R_6, R_{14}, R_{28} – 100 k ohms; R_{27} – 150 kohms; R_{12}, R_{15} – 180 kohms, R_{26} – 200 kohms; R_2 – 510 kohms; R_8 – 680 kohms; R_7 – 820 kohms.

Condensors: C_7, C_8, C_{22}, C_{23} – 100 μμf; C_{11} – 300 μμf; C_1 – 2200 μμf; C_5 – 3300 μμf; C_9, C_{10}, C_{13} – 0.01 μf; C_6, C_{12}, C_{14}, C_{16}, C_{21} – 0.03 μf; C_2 – 0.04 μf; C_4 – 0.05 μf; C_{15} – 5 μf; C_{17}, C_{18}, C_{19}, C_{20} – 10 μf; C_3 – 30 μf; C_{24} – 2000 μf. Tubes: V_1 – 6H8; V_2, V_3, V_4, V_5, V_7 – MTX – 90; V_6 – SG4S; V_8 – 5Ts4S. Relays: R_1, R_2 – 500 ohms. Tr – power transformer. SR – selenium rectifier feeding lamp in counter; input terminals: 1) photocell, 2) counter contacts, 3) windings on pen-recorder in series with separate 60 volt rectifier, 4) lamp in counter; P_1 – "times 2" counting down circuit.

These difficulties were overcome by using a small size resistance-type photocell [1, 2]. The blood passes into the plexiglass vessel(1) through the tube(2) forming a drop on the end (Fig. 1). In falling from here the drop passes in front of the photocell (3), partly cuts off the light beam from the lamp (4), and again passes into the blood vessel along the tube(5). The rubber stopper (6) seals the system hermetically so that it is possible to record blood flow not only in the veins but in the arteries.

When recording venous flow using wide bore rubber tubes and the usual glass cannulae, separation of the blood cells from the plasma may occur. This is due to the slow flow of blood in the wide tubes and the increased sedimentation rate due to adding heparin to the blood. It is therefore important that tubes 2 and 5 should have a diameter approximately equal to that of the corresponding blood vessels. We used polyvinyl chloride or polyethylene tubes, and joined them directly to the blood vessels. Before attaching them the dropping chamber was one-third filled with physiological saline.

The amplifier (Fig. 2) operates as follows. A pulse which occurs when light is cut off from the photocell by a falling drop passes to the grid of the amplifying tube V_1. The latter sends a pulse to the trigger electrode of the cold cathode thyratron V_2, causing it to ignite. The condensor C_6 discharges through it until the potential difference across its plates falls to a value at which the thyratron no longer conducts. The pulse now passes from the cathode of the thyratron through switch (1) in position 1 and is taken to the trigger electrode of a second similar thyratron V_5. This fires causing relay R_1 to operate so that voltage is now supplied to the coils of the electromagnetic pen-recorder through the contact K_1R_1. This voltage is supplied by a separate rectifier connected to the amplifier through the binding posts (3) in series with the windings.

In order to eliminate any systematic error in the pen-recorder caused by a different position of the pen at the end of successive half cycles and differences in the length of the pulse from the counter (1) a circuit for stabilizing the length of the output pulse is included, and this consists of condenser C_{15} and relay R_2. When relay R_1 operates K_2R_1 closes so that C_{15} charges through R_{19} to a potential which operates relay R_2.

When this happens K_1R_2 opens breaking the anode circuit of V_5, which ceases to conduct so that current fails in R_1. Thus the duration of the pulse to the pen-recorder is determined by the time elapsing between the closures of relays R_1 and R_2 and is always equal to the discharge time of condenser C_{15} (0.1 ± 0.01 seconds). Contacts K_2R_2 or relay R_2 allow condenser C_{15} to discharge rapidly, after which the circuit returns to its original condition.

The maximum frequency of impulses which can be satisfactorily recorded is not greater than 5 c/s for a pen velocity of 200 mm/second. This limits blood flow measurement to 18-20 ml/minute (4-5 drops per second), although even at 30-35 ml/minute the drops have not merged into a continuous stream. To record processes occurring at a higher frequency than this, the counting down circuit, composed of the two cold cathode thyratrons V_3 and V_4 is introduced. When switch P_1 is in position 2 only every second input pulse passes through the counting down circuit to the output thyratron V_5 which causes the pen of the recorder to operate. Thus the upper frequency limit is increased to 9 c/s.

If necessary the electromechanical counter EMS, connected through contacts K_3R_2 of relay R_2 may count impulses without them being recorded.

The scope of the apparatus is increased by introducing valve V_7 which allows the recording meter to be operated by a switch connected to binding posts (2). In this way for instance it is possible to use devices operated by waves of arterial pressure which enables uninterrupted recordings of the pulse rate etc. to be made.

The power supply is derived from the mains through the rectifier consisting of V_8 with an RC filter and neon stabilizer L_6.

Tests showed that displacement of the shadow of the drop on the surface of photocell did not affect the operation of the counter. This made its precise positioning unnecessary. Condensation of moisture or blood fragments on the walls of the dropping chamber caused no interference with the recording of the drops. The sensitivity of the circuit was sufficient to record not only blood drops but also transparent liquids such as perfusates. In the latter case direct closure of contacts connected on to binding posts(2) could be used.

Since the dropping chamber is hermetically sealed, the system through which the blood flows constitutes a siphon. The additional resistance to the blood flow consists therefore of that due to viscosity effects in the connecting tubes, and is independent of the height of the counter above the body of the animal. This is very

important in recording blood flow from veins. The small size of the counter facilitates simultaneous registration when recording outflow or inflow of blood to many organs.

SUMMARY

A hermetically sealed photoelectric drop impulse pickup has been constructed for measuring the volumetric blood flow in veins and arteries by means of the pen-recorder. A miniature semi-conductor photoresistance is used in this pickup. The wiring circuit of the electron amplifier which controls the pen-recorder is presented. The amplifier allows the internals to be registered either between each drop or after every drop and automatically controls the displacement of the recorder pen at regular intervals. The amplifier also registers the frequency of mechanical impulses (heartbeat, etc.) by means of the pen-recorder.

LITERATURE CITED

[1] Kolomnets, Resistance-type Photocells,* (1957).

[2] M. S. Sominskii, Resistance-type Photocells,* (1957).

[3] V. M. Khaiutin, Biull. Eksptl. Biol. i Med. 40, 8, 72-75 (1955).

[4] S. M. Hilton, J. Physiol. 117, 48P-49 (1952).

[5] S. M. Hilton and D. W. Lywood, J. Physiol. 1954, Vol. 123, pp. 64P-66P.

[6] P. Lindgren and B. Uvnäs, Acta Physiol. Scand. 1954, Vol. 32, No. 2-3, pp. 259-263.

* In Russian.

APPARATUS FOR MEASURING ARTERIAL AND VENOUS PRESSURE AND TONE

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At the present time many methods are used in studying the cardiovascular system in man and animals.

Any one of these methods measures only a single quantity, which may be the arterial or venous pressure, the temperature, arterial or venous tone, rate of blood flow, etc.

However, frequently the study of only one or two of these quantities is insufficient, while simultaneous use of several different methods, which may be very complex, is difficult or even impossible, particularly when cannulation methods are not applicable.

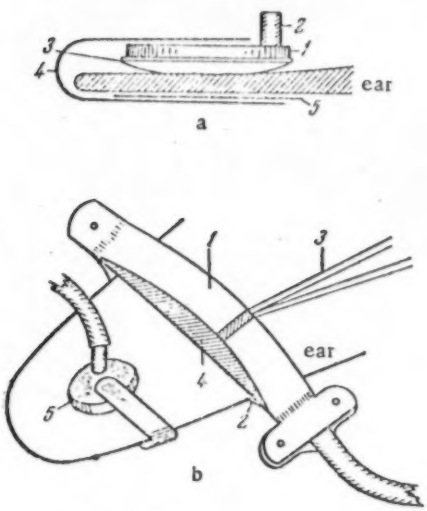

Fig. 1. (a) Spring element; (b) clamping sleeve. Explanation in text.

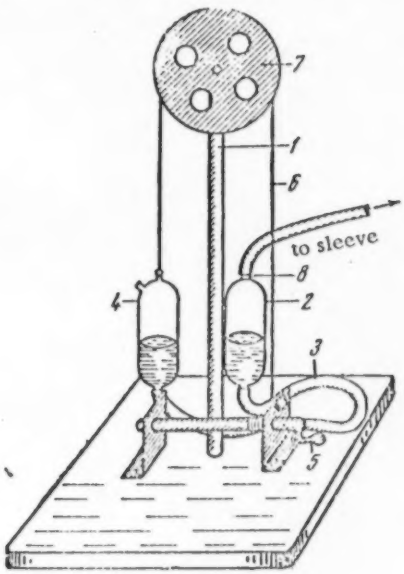

Fig. 2. Apparatus for evenly inflating the clamping sleeve with air. Explanation in text.

Changes in the arterial and venous pressure, arterial and venous tone, and also reflex reactions to compression of an organ can be studied using a volume manometer.

In 1912, Frank and Ray, and Howell devised an apparatus for measuring venous pressure by observing volume changes in the organ under investigation.

In 1930, Ia. S. Leitman produced a modification of this method. The apparatus, which he called a volume-manometer, allows measurement of arterial and venous pressure, rate of blood flow, and elasticity of arteries and

capillaries. In 1940, V. A. Val'dman introduced the plethysmograph method of measuring venous pressure. In 1952 N. I. Arinchin constructed a volume manometer which allowed measurement of arterial and venous pressure and venous tone.

By use of the universal manometer described by us here, it is possible to measure arterial and venous pressure and tone, as well as reflex contraction of the blood vessels in dogs. For our investigation we used the ear flap of a dog. This has two advantages. Since the distal portion of the ear has no muscles, firstly there is no danger of artifacts caused by muscular contraction [1], and secondly one can be sure that it is the skin vessels which are being investigated.

The apparatus consists of three parts for measuring pulse volume, for measuring pressure in the sleeve, and for clamping the ear. The first consists of a plethysmograph which allows the extent to which the ear is filled with blood to be determined. It consists of a spring element (the receiving portion) and a sensitive Votchal-Filippovich capsule (recording portion). The spring element (Fig. 1,a) consists of a flat metal box (1) diameter 3 cm and height 0.5 cm, to the bottom of which a metal tube (2) is soldered. Within the element is placed a phosphorbronze spring (diameter 0.5 mm), consisting of 3-4 turns, one end of which lies at the base of the element and the other against a ring of x-ray film of a somewhat smaller diameter than that of the box. A sheet of thin rubber is stretched over the free edge of the element so that it is pressed out by the spring at the edge (3). The device is fixed to the animal's ear by means of a bent strip (4). To record the pressure in the system we used the contact mercury manometer of P. M. Vinogradov, made at the AMN SSSR Moscow Instrumen. Works. The clamping device consisted of a pressurizing system and a sleeve.

To produce an even increase in pressure in the sleeve, the apparatus shown in Fig. 2 was assembled; this consists of a Bunsen stand (1) to which is attached one of the two communicating vessels (2). The second glass vessel (4) is attached to a cord which passes over a pulley (7) and is attached to the rod which ends in the handle (5). Mercury is poured into both vessels. The output tube (8) on the upper part of the fixed vessel is connected to the rubber balloon (4) (Fig. 1,b). When the handle (5) is turned slowly the cord is wound around the rod and vessel (4) rises. Mercury now passes out of (4) into (2) displacing air from it into the rubber balloon (4) (Fig. 1,b). The clamping sleeve (Fig. 1,b) consists of two metal strips (1 and 2), which enclose the rubber balloon (4) which is connected to the pressurizing mechanism.

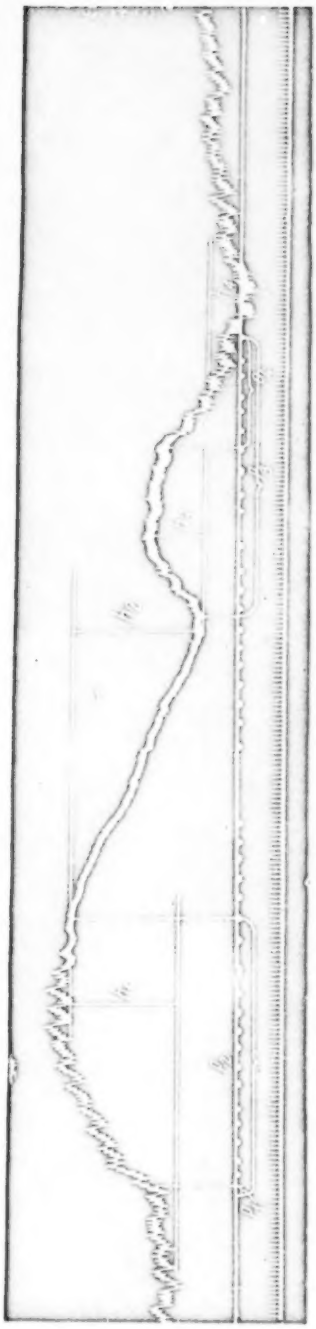

Fig. 3. Trace recorded using the universal manometer. Explanation in text. Reduced three times. Traces from above downwards: plethysmograph, pressure markings, and time markings.

The three traces were recorded on photographic paper by means of an oscillograph. The animals were placed on their sides.

The operations were performed in the following sequence.

The clamp and sleeve were attached to the ear and held in position by the strings (3) (Fig. 1,b). The plethysmograph (5) was placed distally to this. On rotating the handle the air displaced from the fixed vessel passes into the clamped sleeve so that the pressure rises.

A contact manometer gives an electrical pulse to the oscillograph every 5 mm mercury pressure interval, and these are recorded on the moving photographic paper.

The plethysmograph element is sensitive to changes in the degree of filling of the blood vessels produced by pressure applied to the vessels by the clamping device. Figure 3 shows the traces obtained using the "universal manometer."

We agree with the usual view that the pressure corresponding to the first hump of the plethysmogram (P_1) corresponds to the pressure in the veins. The pressure at which the pulse oscillations disappear (P_2) corresponds to the maximal pressure in the arteries. The height to which the plethysmogram trace rises (h_1) indicates venous tone and initial flow rate, and the pulse amplitude is an indication of the tone of the arterial walls. The pressure at which the second elevation of the plethysmogram (P_3) occurs corresponds to the maximum arterial pressure, and P_4 that of the veins on decompression. The heights h_2 and h_3 evidently indicate the tone of the vessels, and the drop (h_4) of the plethysmogram after complete decomposition corresponds to reflex blood vessel contraction.

SUMMARY

A universal manometer which consists of an instrument with the aid of which the pressure that is created in the sleeve is recorded on an apparatus, and of a mechanism for gradual compression of a dog's ear lobe, and which registers the pulse volume is proposed. One may determine the value of arterial and venous pressure by the curve obtained with the aid of this manometer. The tone of the veins and arteries, as well as the reflex contraction of the vessels after decompression may, likewise be evaluated by analyzing this curve.

LITERATURE CITED

[1] V. V. Iakovlev, Biull. Eksptl. Biol. i Med. 40, 8, 69-72, (1955).

A METHOD FOR STUDYING CEREBRAL VASCULAR TONE VARIATIONS IN ANIMALS

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(Received March 2, 1957. Presented by Active Member of the Acad. Med. Sci. USSR S. V. Anichkov)

Up to the present time there has been a dearth of information concerning cerebral blood supply in healthy subjects and its changes under pathological conditions and in response to the action of drugs and to the composition of the inspired air; this is largely due to experimental difficulties.

The methods used at present are not without serious defects. Thus, the best method at the present day, that of the "skull window" suffers from the limitation that only the superficial vessels of the brain and meninges are visible. The gas method of investigating intracranial circulation is not easy. Many of the methods for measuring variations in intracranial pressure or volume associated with changes in the volume of the cerebral vessels, give valuable information about cerebral vascular tone. However the main shortcoming of these methods is that the cranial cavity is no longer hermetically sealed, and this, in the opinion of many authors, causes alterations in intracranial circulation.

These difficulties can be overcome by use of the electroplethysmograph described by A. A. Kedrov and A. I. Naumenko [1]. This method gives a more accurate record of cerebral vascular tone, but also suffers from certain defects; the curve obtained represents not only variations in the volume of the cerebral vessels but also changes in the blood flow in them.

Fig. 1A. Strain gauge assembly for recording intracranial pressure.
1) Metal box enclosing the strain gauge; 2) projection with screw thread; 3) sensitive membrane.

As H. Sigward's [6] calculations showed, rapid (pulse) changes in cranial volume cannot be completely compensated by movement of the fluid. Considering that the brain is situated in a hermetically sealed space with inextensible walls, it is clear that every change in its volume must be accompanied by both a displacement of cerebrospinal fluid and a change of intracranial pressure.

In order to study intracranial circulation in animals, we used the method of recording the pressure variations. While admitting that there are rhythmical variations in the intracranial pressure corresponding to variations in the extent of filling of the blood vessels which in turn are associated with the heart beat and with respiratory movements, we consider these variations to be very small.

Any method used for measuring intracranial circulation must fulfil the following conditions: 1) the receiving device must be sensitive to very small pressure variations;

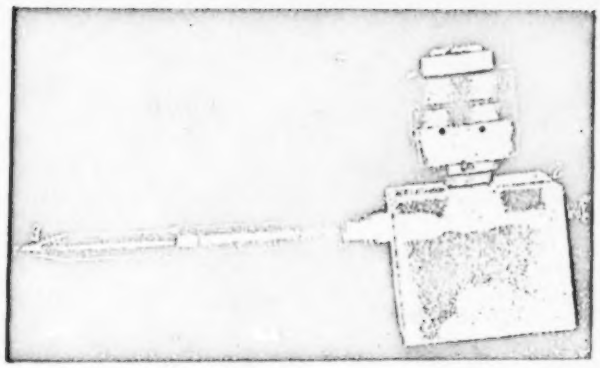

Fig. 1B. Strain gauge assembly for recording arterial pressure.
1) Strain gauge; 2) plexiglass vessel into which gauge is screwed;
3) cannula for insertion into artery.

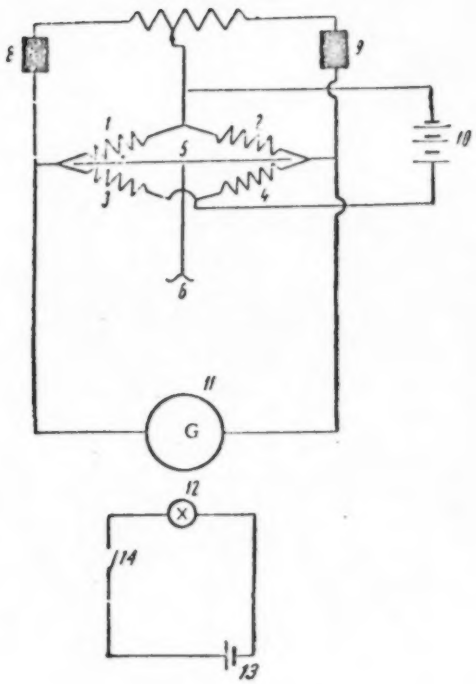

Fig. 2. Diagram of the apparatus.
1, 2, 3, 4) Strain gauge resistances (diameter of wire 0.02 mm, resistance ~ 200 ohms); 5) disk to which stain gauge is fixed; 6) corrugated membrane receiving oscillations; 7) resistance for balancing bridge (10,000 ohms); 8, 9) resistances for balancing bridge (5,000 ohms); 10) 12 volt batteries; 11) short period galvanometer (10^{-9}amp.); 12) galvanometer lamp; 13) 3.5 volt battery; 14) lamp switch.

2) it must be equally sensitive to rapid and slow changes; 3) the recording of the changes must be carried out by a device which is substantially free from inertia; 4) the skull must remain hermetically sealed.

Therefore we chose a method which allows very small variations in pressure to be transformed into voltage variations. Many such transducers are known, including electromagnetic, piezoelectric, etc.

In view of the requirements outlined we decided to use a strain gauge which was prepared for us by S. A. Evdokimov. The gauge had no inertia, it was superior to other devices in that it was equally sensitive to rapid pressure variations and to constant pressure changes. Its sensitivity was very high: it was capable of recording pressure changes equal to a few mm of water, which corresponds to the intracranial pulse pressure variation in experimental animals (cats). The gauge consisted of four wires with a resistance of 200 ohms each connected in a bridge circuit supplied by a 12-volt accumulator. Each resistance was made of a very fine wire of a special high resistance alloy wound to form a so-called sensitive network. The wire was fixed with a special adhesive to the surface of a metal disk. Changes in the shape of the disk caused deformation of the wire and so changed its resistance.

When not deformed the currents through the different arms of the bridge are equal, and the voltage output from the bridge is zero.

Deformation causes resistance changes in the arms, and this leads to potential changes which are proportional to the deforming force, i.e., to the pressure on the gauge.

The apparatus is contained in a small metal box (Fig. 1A and 1B) which ends in the central projection bearing a screw thread 2. This projection acts as a

stopper which screws hermetically into the trepanned opening in the skull. The distal end of the projection is covered with a thin corrugated membrane (f), which is connected by a wire to the bridge network of the strain gauge (Fig. 2). The membrane receives the pressure variations directly and transmits them to the strain gauge where the deformation of the resistance wires causes a voltage change in the network. These are recorded directly by a short period galvanometer on light-sensitive paper.

We used a similar but less sensitive gauge for recording intra-arterial pressure changes by connecting it to a cannula inserted into the artery (see Fig. 1B). Simultaneous recording of intra-arterial and intercranial pressure changes was then possible.

A diagram of the apparatus is shown in Fig. 2.

In acute experiments on cats under medinal anesthesia, using this apparatus, the following curves of arterial and cranial pressure were obtained (Fig. 3). In both of the curves changes associated with pulse and respiration are clearly shown. An increase in pressure corresponds to a movement of the line upwards, and a fall to a movement downwards. The amplitude of the curve depends on the sensitivity of the gauge, and this can be varied by changing the voltage supply to it.

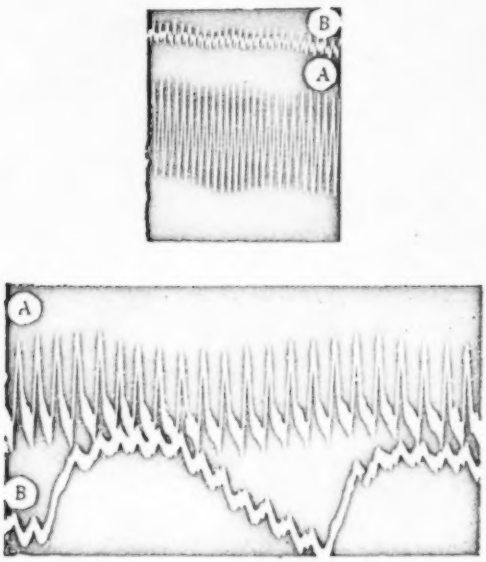

Fig. 3. Curves of (A) arterial and (B) intracranial pressure.

It has been suggested by B. N. Klosovskii [2] that pulsation of the cerebral vessels may occur only when the skull is not hermetically sealed; we therefore made a recording of the intracranial pressure in cats while maintaining the skull hermetically sealed by using the screwed-in gauge, as well as when it was not sealed. Pulse variations were less when the skull was not sealed.

These changes are quite understandable, since when there is an increase in blood flow to the brain during systole, the intracranial pressure increases much more in the case when the brain is unable to expand.

Thus, by using strain gauge recording of variations of intracranial pressure, we have confirmed the results obtained by other methods (electroplethysmograph, piezomanometer), which showed that there are variations in intracranial pressure associated with pulse and respiration, and that both in cerebral and other vessels these variations are due to irregular variations in the circulation.

By using the apparatus described to record changes in intra-arterial and intracranial pressures and their variation in response to pulse and respiration, and by comparing the results, we have been able to determine the variation in cerebral vascular tone under different conditions.

SUMMARY

The level and pulse variations of the arterial and intracranial pressure in animals were studied for evaluation of the changes of the tone of brain vessels which take place under the effect of various pharmacological substances.

Parallel registration of the variations of intracranial and arterial pressure was done by the tensiometric method. A detailed description of the apparatus and the scientific basis for the use of this method are presented in this paper.

It was established that pulse variations of the intracranial pressure which occur as a result of pulsation of the brain vessels appear only in such conditions in which the cranium is hermetic.

The curves of intracranial and arterial pressure of a cat are presented as an example. These curves were obtained under the effect of substances which greatly reduce the tone of the brain vessels (carbon dioxide) or those which increase it (oxygen).

LITERATURE CITED

[1] A. A. Kedrov and A. I. Naumenko, Problems of the Physiology of the Intracranial Circulation and Their Relevance to Clinical Practice,* Experimental investigation, Leningrad 1953.

[2] B. N. Klosovskii, Circulation of Blood in the Brain,* Moscow 1951.

[3] Iu. Iu. Kolchinskii, I. G. Korovianskii and V. A. Orlov, Zavodskaia Laboratoriia, 12, 6, 7-8 (1946).

[4] Kedi, Piezoelectricity and Its Practical Application,* Moscow 1949.

[5] M. E. Marshak, Piezoelectric Transducers for Recording the Pulse in the Vessels of the Finger and the Lobe of the Ear. Biull. Eksptl. i Med. 5, 75-77 (1956).**

[6] H. Sigward, Langenbecks Arch. and Deut. Z. Chir. 1954, Vol. 278, No. 2, pp. 115-122.

* In Russian.

** Original Russian pagination. See C. B. Translation.

A CLINICAL METHOD FOR INVESTIGATING HIGHER NERVOUS FUNCTION TESTS FOR GENERALIZING ABILITY

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Clinical tests for higher nervous function are usually carried out by establishing conditioned reflexes, i.e., by associating previously indifferent stimuli with others which elicit a certain reaction. However, a feature of human reactions is that they are not limited to previously encountered stimuli associated with this or that event. A man can react adequately in new conditions in which he finds himself for the first time, i.e., he reacts correctly to new stimuli; all that is necessary is that the new stimuli should constitute a part of some group of stimuli of which he has encountered examples previously. Thus a man may react to a whole group of related stimuli in a way which was developed as a response to only certain members of the group. In this way he shows his ability to generalize from his acquired experience.

Psychological methods of investigating all aspects of generalizing ability do not allow the different experimental factors or the results to be expressed quantitatively, as in the case of a physiological investigation.

The following is a method of investigating the ability of a man to extrapolate onto a whole group of stimuli a particular type of reaction which was elaborated to some members of the group only.

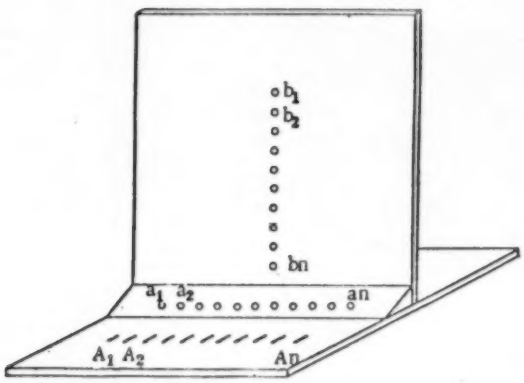

Diagram of the apparatus for investigation of higher nervous function (apparatus drawn turned towards subject).

A vertical row of lamps (b_1, $b_2 \ldots b_n$) is placed in the subject's field of view (see drawing). Below this a horizontal row of lamps (a_1, $a_2 \ldots a_n$) is arranged. Opposite each lamp of the horizontal row there is a button (A_1, $A_2 \ldots A_n$). The experimenter can light any lamp in either row a or row b.

The subject is told that as soon as any lamp in the horizontal row lights he is to press the corresponding button. A control lamp facing the subject indicates whether he has done this correctly or not. After the instructions have been given, lighting any lamp in a row results in the corresponding button in row A being pressed, i.e., a reaction of the type $a_i \rightarrow A_i$,* so that lighting lamp number i causes button i to be pressed, whereas lighting a lamp in row b does not lead to a button being pressed ($b_i - / \rightarrow A_i$). When the experimenter is convinced that the subject has properly understood his instructions, the experiment is started.

Before the experiment, and without telling the subject, the experimenter sets up a certain set of connections (correspondences) between rows b and a, for instance b_1 corresponds to a_1, b_2 corresponds to $a_2 \ldots b_n$ to a_n; or b_1 corresponds to a_n, b_2 corresponds to $a_{n-1} \ldots b_n$ corresponds to a_1; or b_1 corresponds to a_1, b_2 corresponds to a_n, b_3 corresponds to a_2; b_4 corresponds to a_{n-1}, etc. Any degree of complication of the connections between row b and row a is possible.

Let us consider the course of one simple experiment, the first of those described above. The experimenter switches on one of the lamps in row b for instance b_3, and immediately after it – the corresponding lamp in row a – a_3. The subject responds to this by pressing button A_3. A similar process occurs with the other lamps, and there is no regular sequence in the order in which the lamps are lit (the lighting sequence may be chosen from a table of random numbers). However not all the lamps are involved in these sequences of the type ($b_i a_i \rightarrow A_i$). The choice by the subject of the correct button is reinforced by the word "correct."

After a certain number of sequences a conditioned reflex is developed: the subject now responds to pressing the button in response to the lighting of a lamp in row b only, this lamp having been previously associated with one in row a ($b_i \rightarrow A_i$). He does not wait for a lamp in row a to light. At the same time if a lamp in row 'b' not previously associated with one in row 'a' lights, it does not cause the corresponding button to be pressed ($b_m - / \rightarrow A_m$). However after a large number of sequences of the type $b_i a_i \rightarrow A_i$ the subject will press button A_m in response to the lighting of a lamp b_m which had never previously been associated with the lighting of a lamp in the other row. Thus a reaction of the type $b_i \rightarrow A_i$, developed in response to certain of the lamps, becomes extrapolated to include the whole group. The rate at which this reaction is elaborated depends upon higher nervous function and individual characteristics as well as on a complexity of the connections between lamps a and lamps b.

The course of an experiment can be represented diagramatically as follows:

$a_i \rightarrow A_i$
$b_i - / \rightarrow A_i$
$b_i a_i \rightarrow A_i$
$b_i a_i \rightarrow A_i$
} Initial position

$b_i \rightarrow A_i$
$b_m - / \rightarrow A_m$
} Conditioned reflex developed, but no extrapolation

$b_i a_i \rightarrow A_i$
$b_m \rightarrow A_m$
} Extrapolation

The ability of the subject to generalize is indicated by the number of sequences of the type $b_i a_i \rightarrow A_i$ required before the reaction of the type $b_m \rightarrow A_m$ occurs.

When the reaction of the type $b_m \rightarrow A_m$ has been established firmly, it is possible to follow this by investigating the degree of perseverance of the subject. For this the association of lamps in row b and row a is changed in some way, for instance b_1 is made to correspond to a_n, b_2 with $a_{n-1} \ldots b_i$ with $a_{n-1+1} \ldots b_n$ with a_1. One can then find how rapidly, i.e., after how many sequences the subject stops pressing the button which

* The following abbreviations are used: a_i – lighting lamp number i in horizontal row; b_i – lighting lamp number i in vertical row; A_i – pressing button number i.

corresponds with the reaction $b_m \rightarrow A_m$ and changes to the new reaction $b_i \rightarrow A_{n-i+1}$ and therefore to $b_m \rightarrow A_{n-m+1}$. It must be realized that the stability of the reaction $b_m \rightarrow A_m$ depends also on the number of sequences $b_i a_i \rightarrow A_i$, which have been given after elaborating the reaction $b_m \rightarrow A_m$. These additional sequences $b_i a_i \rightarrow A_i$ build up a characteristic "stability reserve reaction."

Thus this method we have proposed enables the ability of the subject to generalize and to extrapolate his reaction to be determined quantitatively under the conditions of a physiological experiment.

Experiments have shown that in healthy subjects, generalization of the simplest type (sequence $b_i a_i \rightarrow A_i$) occurs after 3-6 sequences. In oligophrenics there is no generalization even after several dozen sequences.

SUMMARY

The method which is described in this paper gives the opportunity not only to trace the formation of conditioned reflexes in man but also to measure the rapidity of transfer of a correct reaction formed to several stimulants of a definite series to all stimulants of this series (generalization, extrapolation of reactions). A subject under investigation should press knob A_i when the lamp a_i is lit (look at the scheme). Then after several associations of switching on b_i and a_i lamps, a healthy individual who is examined presses the knob A_i, when lamp b_i is lit (conditioned reflex). He presses knob A_m when the lamp b_m, which was never lit before is switched on (extrapolation of reactions). The latter type of reaction could not be formed in oligophrenics.

A METHOD OF MEASURING PULMONARY EXCRETION

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In studying pulmonary excretory function and its regulation at the suggestion of N. P. Sinitsyn, we encountered many experimental difficulties. Existing methods of measurement of pulmonary respiration do not enable the amount of volatile substances excreted through the lungs to be measured. The commonest methods use the closed circuit system with constant circulation of air (Benedict [1], Knipping [2] and others). Compensation for air movements due to respiration is afforded in these systems by a spirometer which may contain many materials which absorb the volatile substances excreted by the lungs. This must certainly cause errors, particularly when it is realized that large quantities of air circulate through the absorbers.

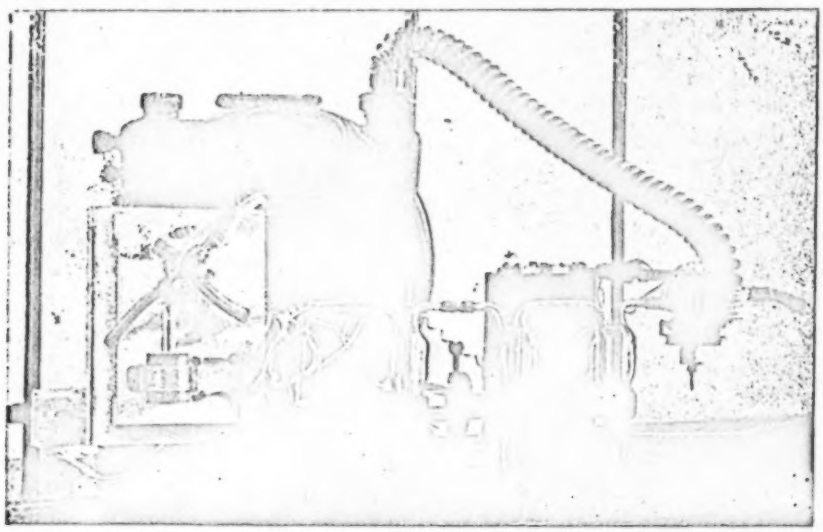

Fig. 1. General view of the apparatus for studying pulmonary excretion.

We have worked out a method of breathing directly through the absorbers [3], but in many cases it cannot be usefully applied for the investigation of pulmonary excretion of substances which are not readily absorbed. For complete absorption of these substances the amount of absorbent substance required becomes so great as to cause a considerable resistance to the movement of the expired air, so that the conditions become nonphysiological. We have therefore devised a new method for the investigation of pulmonary excretion, which is in large measure free from these shortcomings. The basis of the method is that of supplying auxiliary suction through the absorbers timed to coincide with expiration.

The whole setup, as shown in Fig. 1, consists of a gas flow meter to measure pulmonary ventilation, a box containing valves, a capsule which makes and breaks a contact, a three way solenoid operated tap, and a suction pump (not drawn).

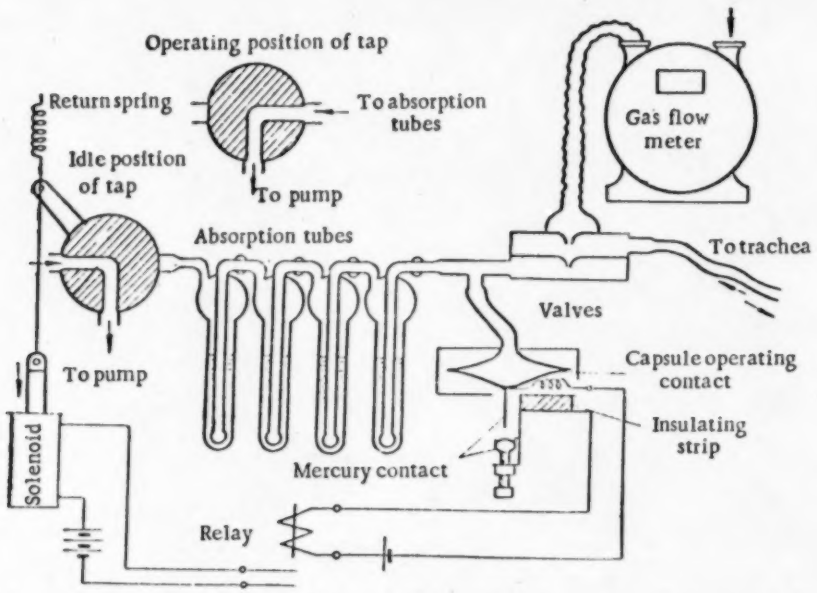

Fig. 2. Diagram illustrating method of investigating pulmonary excretion (description in text).

The operation of the system is as follows (Fig. 2). At expiration the pressure in the absorbing system rises; this causes the capsule to expand, to close the mercury contact so that the relay included in this circuit operates and energizes the solenoid. The armature of the solenoid is pulled into the coil and so operates the three way tap connected to the suction pump, placing it in the working position. The pump which is always working now begins to suck air out of the absorbers. As soon as the pressure in the system falls to atmospheric level, or as soon as inspiration starts, the contact on the capsule rises so breaking the mercury switch. As a result the relay and solenoid circuits are opened, and the return spring puts the three way tap back into the off position. The additional suction through the absorbers stops, and the pump sucks in air from outside.

An important detail which to a large extent determines the precision of the apparatus is the capsule, which must react to very small pressure changes within the system. It is best made out of two springy rubber disks of diameter about 5 cm stuck together round the edge. The hole is made in one of these disks for connection to the absorbing system, while a silver electrode for the mercury contact is secured to the other disk. The capsule together with the adjustable mercury contact are mounted in a metal case.

A telephone relay, whose windings are of thicker wire so that it can work from a potential of 3-4 volts is used to switch in the solenoid which takes a heavy current. The construction of the tap and the dimensions of the solenoid will be determined by what is available; it is important that the movement from the idle to the working position and back should be as rapid as possible. The type of suction pump will depend on the species and size of the experimental animal. For rabbits and small dogs a filter pump works well.

The results of the investigation of pulmonary excretory function are shown in Fig. 3.

It should be possible to use this method for studying pulmonary excretion in human objects.

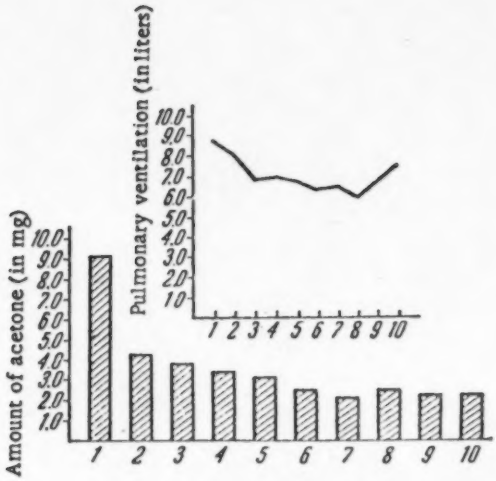

Fig. 3. Excretion of acetone through lungs in rabbit after intravenous injection of 0.5 ml per kg weight.

SUMMARY

A method of study of the excretory function of the lungs was elaborated, which allows registration of the amount of substances excreted by the lungs.

The principle of this method consists in certain of auxiliary draught air movement through an absorber which combines the volatile substances only at the moments of expiration.

A description of this installation is presented.

LITERATURE CITED

[1] Francis G. Benedict and W. E. Collins, The Boston Med. a Surg. J. 1920, Vol. 183, pp. 449-458.

[2] Knipping – cited in Sterling's Principles of Human Physiology, Philadelphia, 1936.

[3] E. G. Iziumov, Farmakol. i Toksikol 5, 23-27, (1955).

A MODIFIED PRESSURE CHAMBER FOR USE IN A SMALL LABORATORY

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In working on the problem of oxygen lack, many difficulties are encountered such as failure to obtain a pressure chamber of suitable size, difficulty in recording rate of "ascent" and the degree of rarefaction of the air, etc. We thought that our arrangement of a pressure chamber would be of interest to other investigators.

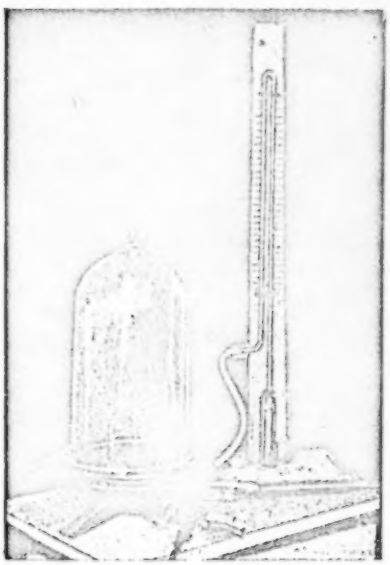

Fig. 1. I. B. Friedland's pressure chamber.

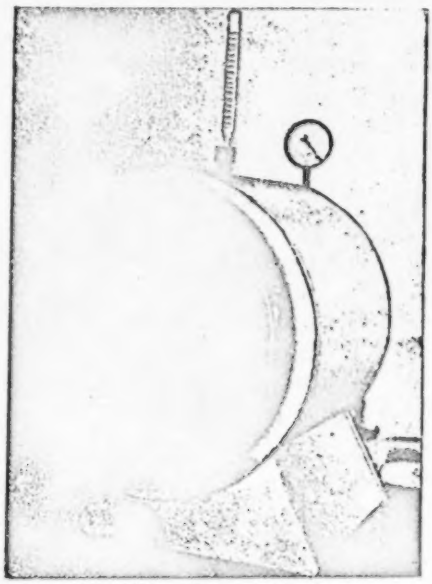

Fig. 2. Vacuum drying cupboard type VSh-0035M.

At first we used a pressure chamber of the type proposed by I. B. Friedland (Fig. 1). Upon a cast iron base there is mounted a 45 cm diameter disk in which there are holes for the supply and withdrawal of air; a rubber ring is placed on the disk and a glass bell jar with a broad lower edge placed upon it. Air is removed by means of a suction pump. Fresh air is continuously supplied to the chamber. The pressure is recorded by a mercury manometer.

This chamber was not entirely adequate for our purposes, as only one animal of average size such as a cat or rabbit could be placed in it. We therefore constructed a special pressure chamber. The basis of this was the vacuum drying cabinet, type VSh-0035M (diameter – 364 mm, length – 400 mm; Fig. 2).

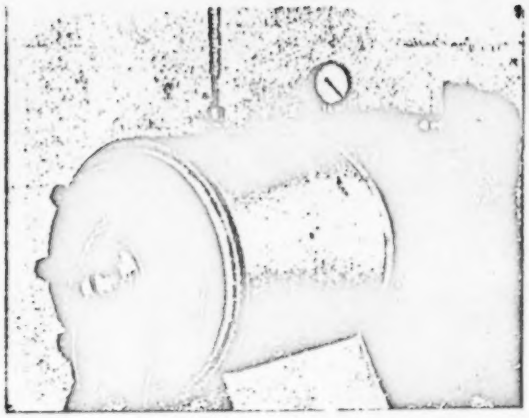

Fig. 3. Vacuum drying cupboard modified for use as pressure chamber.

Many alterations to this cabinet were effected (Fig. 3). A piece of transparent Stalinite type glass 7 mm thick and 150 mm diameter interleaved with a layer of plastic was mounted hermetically on the outside of the middle of the roof. A needle valve, previously in the middle of the roof was moved down to the bottom. Leads to a 6 volt lamp for lighting the chamber were taken in through the top of the roof.

For sucking the air out of the chamber, we used a vacuum pump type VN-461 connected to the chamber through a glass oil trap. At all junctions in the system, vacuum type screw connections were provided. A constant flow of fresh air into the system was obtained through the needle valve.

Three pipes were fitted to the place previously occupied by the dial type vacuum meter, and they were connected to the following pieces of apparatus: a dial type vacuum meter, a membrane variometer, and an aneroid altimeter with two pointers.

The original vacuum meter indicated the pressure in mm of mercury. The two pointer aneroid altimeter indicated "height" in meters and kilometers. The membrane variometer gave a precise indication of the rate of rarefaction, and showed the rate of "rise" in meters per second.

After the predetermined height had been reached a "platform" was established, and the pressure was maintained at a constant level by equalizing the amount of air withdrawn to that entering. The animals could be observed during the experiment through an inspection window.

SUMMARY

A modification of a pressure chamber, convenient for work with rabbits, cats and guinea pigs was proposed. A vacuum drying cabinet of VSh-0035M type was used for the equipment of the pressure chamber. The rapidity and the degree of air rarefaction was registered in a chamber by 3 instruments: by a vacuum meter with a pointer, by a membrane variometer and an aneroid altimeter. A special inspection window permits observation of the behavior of laboratory animals.

A METHOD FOR THE SEROLOGICAL INVESTIGATION OF DESOXYRIBONUCLEOPROTEINS*

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Desoxyribonucleoproteins are an important component of living cells and tissues and play an essential role in many vital processes. The participation of nucleoproteins is especially great in processes of cell division and synthesis of albumins. Because malignant tumors have intense protein synthesis, the study of nucleoproteins in association with the cancer problem assumes especially great significance.

It is well known that various proteins can be studied within the organs and tissues of man and animals by numerous methods such as complement fixation, reaction of agglutination, precipitation, etc. In the investigation of desoxyribonucleoproteins, however, there is a difficulty associated with the fact that such substances are insoluble in physiological saline solution.

The desoxyribonucleoproteins dissolve either in an extremely low ionic concentration or a very high concentration, i.e., either in distilled water or in NaCl solution of 1-2 M strength. In physiological saline solution, the desoxyribonucleoproteins (DNP) precipitate as fine threads.

It is for this reason that we undertook the task of developing a method for the serological study of DNP [1], from the considerations just given.

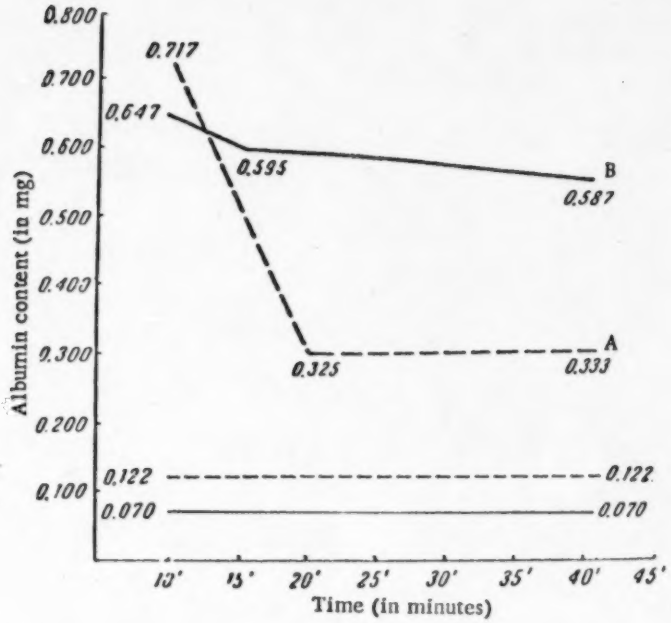
Any tissue that can be thoroughly saturated with DNP dissolved in 1 M solution of NaCl will be termed "adsorbent." Such adsorbents can be transferred into a solution of physiological saline and the DNP fixed firmly to its surface. As the adsorbent we employed "Whatmann 1" papers, filter paper, and also ordinary cotton cloth. Such an adsorbent, saturated with DNP, served us as the test antigen in the immunological reaction.

The results of the immunological interaction between the adsorbed nucleoprotein and the antiserum were judged by the increase in the amount of nitrogen which occurred as a consequence of the protein binding by the antiserum. This is the first time that such an immunological procedure is described in the literature.

EXPERIMENTAL METHODS

Preparation of test antigen DNP. Nuclear DNP was obtained for our experiments by separation from cancer stomach tissues taken from human stomachs removed by surgery. Immediately after the operation, the cancer tissue was frozen, dry ice being used in many instances. The tissues were finely sliced and washed with cold physiological saline in order to remove blood. Then the tissues were homogenized for 7-10 minutes in cold physiological saline to which an inhibitor was added at the first extraction, this agent being a 0.01 M solution of sodium desoxyribonuclease citrate. Further treatment followed the method of A Mirsky and A. Pollister [2].

* This work was presented December 11, 1956 at the Scientific Conference devoted to methods of utilizing the immunological approaches to cancer problems, the gathering being held under the auspices of the Institute of Experimental Biology of the Academy of Medical Sciences of the USSR. See the program of the conference.



Rinsing of loosely held albumins of sera with DNP adsorbed upon cotton tissues (in mg of albumin determined from the nitrogen).
A) Adsorbent + DNP – normal rabbit serum; B) adsorbent + DNP + human antigastric cancer serum.

The tissue remaining after the separation of the cytoplasmic proteins was subjected to prolonged extraction with 1 M NaCl, the process being continued for 2-3 days until a viscid solution was obtained. The undissolved tissue was separated by centrifuging.

The DNP thus obtained was purified by repeated reprecipitation the method used being dilution of the solution from 3 to 8 times by the use of six volumes of water. All the work done on separating the DNP was conducted at temperatures between 2 and 4°.

The DNP preparations so made were mineralized with sulfuric acid and the nitrogen was determined by Conway's method, while total phosphorus was determined by the method of Fiske-Subbarow. The ratio of nitrogen to the total phosphorus varied from 3.6 to 4.3 depending on the number of purifications.

Definite weighed amounts of adsorbent paper or cotton cloth, measured in quantities of 15-60 mg on torsion balances, were immersed for 20 minutes in the cooled solution of DNP dissolved in 1 M NaCl. Special experiments had taken into account the time factor (from 20 minutes to 21 hours) and had shown that DNP adsorption upon the materials was complete within the 20 minutes. In order to fix the DNP firmly upon the adsorbents and then the remove DNP material loosely precipitated, the adsorbing tissues were then transferred for 20-25 minutes into physiological saline. As a rule, the DNP became adsorbed upon the tissues very firmly. The amount of DNP fixed upon the tissues was a function of the surfaces of the adsorbents being used and their concentrations within the solution. The extent of DNP adsorption was determined using the nitrogen method of Conway. The basic potion of our experiments was done with test antigens containing from 0.052 up to 0.122 mg of protein as determined by the nitrogen content.

Preparation of Antisera. We obtained antisera by means of intraabdominal immunization of some of our rabbits by weighed quantities of malignant cancer tissues taken from surgically removed specimens of human stomachs. The remaining rabbits were immunized by homogenates taken at autopsy from normal human stomachs. The injections were done every 3 to 4 days for a total of 24 days, increasing amounts of protein (determined from the nitrogen) being used. The rabbits were exsanguinated 8 days following the last immunization.

The arrangement of the serological reaction specifically fixing the nitrogen. On hollowed glass slides there would be placed the adsorbents impregnated with the DNP. To this there would be added the undiluted immune sera in amounts of 0.2 to 0.4 cc depending upon the weight of the adsorbent. The adsorbents, saturated with the DNP, were incubated with the sera in a moist chamber at room temperature for 1 hour. During the incubation period, the specimens were turned 3-4 times. Then the specimens were placed for 25-30 minutes in physiological saline solution at room temperature. After washing, the specimens were transferred to Kjeldahl flasks for incineration. As a catalyzing agent, hydrogen peroxide was used.

EXPERIMENTAL RESULTS

The figure presents data obtained as a result of rinsing off the lightly held proteins from the sera with DNP adsorbed upon the cotton tissues.

These data indicate that the tissues adsorb and firmly hold a definite amount of desoxyribonucleoprotein which cannot be removed by rinsing. The amount of protein held by adsorption was practically the same after a 10-minute rinsing as it was after 48 hours of rinsing.

Additional Increment of Protein (expressed in mg according to the nitrogen) On Paper Antigen Saturated with DNP Taken from Human Gastric Cancer Incubated with Both Normal and Anticancer Sera

No. of experiment	Adsorbent A	A plus DNP	A plus DNP plus normal rabbit serum	A plus DNP plus anti-normal stomach serum	A plus DNP plus anti-gastric cancer serum
60a	0.017	0.105	0.385	—	0.560
60b	—	0.105	0.385	—	0.770
63	0.020	0.122	0.192	—	0.280
64	—	0.087	0.262	—	0.437
67	0.020	0.122	—	0.840	1.102
69	—	0.140	0.542	0.542	0.717
70	0.012	0.052	0.170	0.227	0.350
71	—	0.087	0.271	0.262	0.385
72a	—	0.073	0.233	0.233	0.467
72b	—	0.073	0.233	0.233	0.467

After the tissue saturated with nucleoproteins was incubated with normal or immune sera, the quantity of protein in the adsorbents increased markedly as a result of fixation of both specific and nonspecific serum proteins. However, as was to be expected, rinsing demonstrated that specific fixation of proteins by antibodies was a far firmer bond than nonspecific association of proteins with normal sera.

The quantity of protein in samples labeled "adsorbent plus DNA plus normal rabbit serum" after a 10 minute rinsing with physiological saline was 0.717 mg; after 20 minutes there was 0.325 mg of protein after further rinsing, no more protein could be removed from the adsorbent. A 20-minute rinsing removed 56% of the protein in the indicated samples.

When samples labeled "adsorbent plus DNP," (incubated with anticancer serum) were tested, it was determined that the 15-minute rinsing diminished the albumin content only by 8% (from 0.647 to 0.595) and that further rinsing removed no more albumin from the adsorbent. This must signify that the nucleoprotein adsorbed on the tissues after incubation with the corresponding antiserum, becomes quite firmly attached to the protein of the antiserum, which must then have a specific character.

In order to have comparable conditions, the specimens labeled "adsorbent plus DNP," after incubation with the normal as well as with the specific antiserum, were all rinsed for 25-30 minutes. When the specimens were rinsed after incubation with cold physiological saline solution, the amount of protein fixed was twice that

obtained by rinsing with physiological saline at room temperature. These experimental relationships were maintained rigorously.

The table presents the data of some of the experiments which demonstrate the amount of nitrogen increase in tissue paper and cotton cloth adsorbents saturated with DNP taken from human gastric cancers after being incubated with normal rabbit sera, with rabbit anti-normal stomach sera and with antisera prepared against human gastric cancers.

This table demonstrates that when adsorbed DNP taken from human cancers is incubated with anticancer sera, a definite specific fixation of proteins takes place; this phenomenon is not observed when the same DNP is incubated with normal sera or with antisera prepared against normal human stomachs. The last finding may be explained by the fact that we were forced to use autopsy material from which to prepare tissues of normal human stomachs with which rabbits were immunized.

It is well known that autopsy material will not yield native DNP because of its destruction. The DNP we extracted from the autopsy material apparently failed to cause the formation of specific antibodies as was the case against the high-polymer native DNP obtained from the surgical specimens.

Thus, when DNP loaded upon adsorbents along with the corresponding antisera is incubated, there is a surface, very firm fixation of the proteins of the specific sera which may be measured quantitatively by the nitrogen which has been fixed. This quantitative method of studying the DNP adsorbed upon cotton tissues permits an investigation of the immunologic specificities of DNP having varying origins. The method is quite simple and lends itself to ready repetition.

SUMMARY

A new method for the quantitative determination of desoxyribonucleoprotein adsorbed upon cotton materials and treated by various immunological procedures is presented.

This method has been used to demonstrate that human gastric cancers produce antibodies which can be fixed by the appropriate specific immune serum.

The procedure is described in some detail. The inherent simplicity of the procedure is emphasized.

LITERATURE CITED

[1] V. S. Gostev, D. G. Grigorian, N. A. Shagunova and N. M. Teplova, Annotations of the Scientific Studies of the Acad. Med. Sci. USSR for 1955, No. 1, 239-241.

[2] A. Mirsky and A. J. Pollister, J. Gen. Physiol., 1946, Vol. 30, pp. 117-143.

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE IN ENGLISH TRANSLATION

January, 1958

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ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Eksptl. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratorii	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

* LIBRARY OF CONGRESS TRANSLITERATION SYSTEM.

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Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiia	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiia	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiia, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Eksptl. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimiia	Uspekhi Biokhimiia	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiia	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiia	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariia	Veterinariia	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniia	Voprosy Pitaniia	Problems of Nutrition
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Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
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